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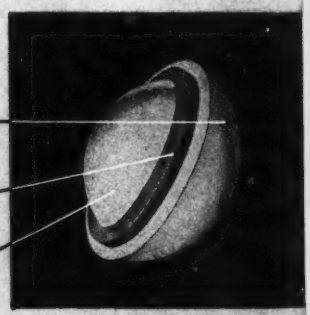

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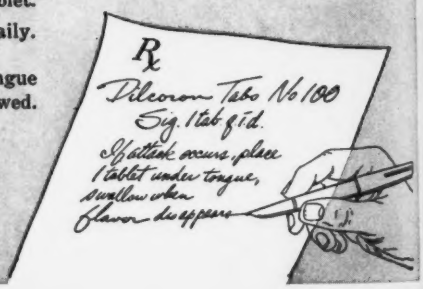

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AN OFFICIAL JOURNAL of the AMERICAN HEART ASSOCIATION

Editorial

Hypertension and Vascular Disease

IT HAS been shown[1] that a functional alteration of the circulation of both kidneys of animals, produced by constriction of the main renal arteries, results in the development of persistent hypertension. Constriction of only one main renal artery is also followed by the development of elevated blood pressure; in the dog, this does not persist for more than about 6 weeks, but in the rabbit, rat, sheep, and goat, it may remain elevated for a long time. The intrarenal hemodynamic disturbance that results from constriction of a main renal artery is undoubtedly identical with the effect, in man, of hypoplasia or obliterative sclerosis of a main renal artery, or compression of the artery from any extraneous cause (tumor, inflammation, or kinking). More significant, however, is the fact that this experimentally produced disturbance of the circulation of the kidney probably simulates the effect of *intrarenal,* obliterative, arterial and arteriolar sclerosis, the condition almost invariably found at autopsy in cases of human, so-called essential hypertension. About this condition the important questions that still remain unanswered are (1) which comes first, the hypertension or the vascular disease, and (2) does the vascular disease play a part in the development of the hypertension only if and when the kidneys are sufficiently affected.

Naturally enough, had I not believed that the obliterative vascular disease within the kidney, or any other pathologic process that produces the same effects on the circulation of the kidneys, precedes the development of the hypertension, it never would have occurred to me to try to produce experimental hypertension by constricting the main renal arteries. Undoubtedly the best evidence brought forward so far in favor of the view that the renal disease precedes the development of the hypertension is the demonstration that the experimental renal hypertension produced by the constriction of the main artery of only one kidney returns promptly to normal when the clamp is released or removed, or when the kidney is excised.[1] Similarly, the finding, now well established,[2, 3] that human essential hypertension associated with obliterative unilateral renal vascular disease, or any lesion that can produce a similar disturbance of the renal circulation, e.g., unilateral chronic pyelonephritis, is cured by the removal of the diseased kidney, is incontrovertible evidence that the kidney does play a primary part in the origin of the hypertension.

Even before, and certainly since, the days of Clifford Allbutt,[4] it has been asserted by many authors that the vascular disease in the kidneys and other organs of individuals with essential hypertension (hyperpiesia of Allbutt) is *caused* by the elevated blood pressure, which is of unknown origin. Although the vascular system of dogs with longstanding (8 years) experimental renal hypertension has been studied with great care, neither simple

intimal arteriosclerosis nor hyalinizing arteriolar sclerosis has been observed as yet in any organs or tissues of these animals, when the hypertension was unaccompanied by significant impairment of renal excretory function. In dogs with experimental renal hypertension of very short duration (just a few days) but with profound impairment of renal excretory function, a widespread arteriolar lesion develops that is indistinguishable from the necrotizing arteriolar lesion of human malignant hypertension. This lesion is fibrinoid arteriolar *necrosis*, but some authors,[5] unfortunately, have been referring to it as arteriolar *sclerosis*, and this has resulted in considerable confusion. It is not sclerosis, and it should not be confused with the hyaline arteriolosclerosis of the benign phase of human essential hypertension.

In recent years, also, there has arisen a false concept of the nature of the pathologic process in periarteritis nodosa. This has resulted mainly from the studies of experimental hypertension in the rat, produced by a variety of means, including constriction of the main renal artery, the production of perinephritis by a perirenal membrane (collodion, silk, cellophane) and the parenteral administration of desoxycorticosterone acetate, with the ingestion of large quantities of sodium chloride, usually in unilaterally nephrectomized rats. The rat is unusually susceptible to periarteritis nodosa. It has been observed[6] in about 15.7 per cent of old but otherwise normal rats, while in the dog it is a rarity. I have seen it only twice, at autopsy, in about 1,500 dogs, and then only the healed lesion, which, in the larger arteries, is almost indistinguishable from healed obliterative fibroelastosis, or severe, simple intimal arteriosclerosis. It was present only in the coronary arteries of these 2 animals. It has been induced in a high percentage of rats even by high-salt intake alone, usually in unilaterally nephrectomized animals.[7] Periarteritis nodosa and typical arteriolar necrosis have been observed together[8] in rats with experimental renal hypertension, but the necrotizing lesion occurred early, and only when there was impairment of renal excretory function, while the periarteritis occurred later, and its development was independent of renal excretory functional disturbance.

That periarteritis should be confused with arteriolar necrosis is understandable, even though they are probably different. Periarteritis affects the larger and small arteries, especially the mesenteric arteries, more commonly than the arterioles, often with development of multiple aneurysm, while the necrotizing lesion of the malignant phase affects only the smallest arteries and arterioles, never the larger arteries, in either man or animal. In periarteritis, the dominant pathologic process is perivascular fibroblastic proliferation, inflammatory cellular infiltration, and subendothelial hyaline plasmatic infiltration, while the vascular lesion of malignant hypertension is characterized mainly by fibrinoid necrosis, often exclusively that, or with only slight perivascular inflammation. When periarteritis does involve the smaller arterial branches, and especially the arterioles, then it may resemble the necrotizing arteriolar lesion, especially if the adventitial proliferation and perivascular cellular infiltration happen to be slight. Finally, the fact that periarteritis nodosa may occur naturally and has been produced in rats and other animals by the injection of tissue extracts, by specific infections, by the injection of horse serum, and by a variety of other means that have not involved the development of hypertension, while the necrotizing arteriolar lesion occurs only when there is hypertension, should be reasons enough for regarding periarteritis as a separate and distinct lesion, and not as has been suggested,[9] as the counterpart in the rat of the arteriolar necrosis of malignant hypertension in man.

Although there appears to be some basis for confusing periarteritis nodosa with arteriolar necrosis, yet there is no reason whatsoever for confusing it with the simple hyaline arteriolar sclerosis that is so characteristic of the benign phase of human essential hypertension. Nevertheless, this is exactly what has

been happening, for some investigators,[10, 11] by referring to periarteritis nodosa, and even to the necrotizing lesion, simply as *vascular disease*, and to the kidneys the seat of periarteritis as *nephrosclerotic* have been implying that they have duplicated the lesions that are characteristic of the benign phase of essential hypertension. My contention is that this is erroneous and misleading, and that, up to the present time, the hyalinizing arteriolar sclerosis and the typical nephrosclerosis that characterize the benign phase of essential hypertension have not been experimentally produced. Certainly, the implication[5] that hyaline arteriolar sclerosis is the end stage of previously existent arteriolar necrosis is, in my opinion, unjustified. As I have stated in previous communications, the problem of the pathogenesis of this type of vascular disease still remains the outstanding one in the possible elucidation of the origin of benign essential hypertension. This is not to say that the study of the pathogenesis of periarteritis nodosa and of arteriolar necrosis should not be pursued, but it is a plea to experimenters, for the present at least, to regard these lesions as distinct and certainly different from the simple intimal arteriosclerosis and hyaline arteriolosclerosis of the benign phase of essential hypertension.

HARRY GOLDBLATT

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To be oriented is to know where to look for the Dawn.—ALAN GREGG, M.D. *Challenges to Contemporary Medicine.* New York, Columbia University Press, 1956, p. 120.

High Serum Transaminase Activity in Heart Disease

Circulatory Failure and Hepatic Necrosis

By THOMAS KILLIP III, M.D., AND MARY ANN PAYNE, M.D.

IN THE PAST few years, determination of the serum or plasma activity of predominantly intracellular enzyme systems has become a valuable adjunct in the study of disease. Since the original report of LaDue et al.[1] many groups have investigated the levels of serum glutamic oxalacetic transaminase activity in a wide variety of conditions. Both myocardium and liver contain high concentrations of this enzyme, and clinical experience has demonstrated the value of repeated determinations of blood transaminase activity in the diagnosis and evaluation of myocardial infarction and liver disease.[2]

In animals an uncomplicated acute myocardial infarction is associated with increases in serum glutamic oxalacetic transaminase activity that correlate roughly with the amount of infarcted muscle.[3-6] In the majority of patients suffering from an acute myocardial infarction, the changes in blood enzyme activity resemble those produced in the experimental animal; after the first day, the level increases, usually to less than 400 units, and then gradually falls to normal within a 3- or 4-day period.[7] Infrequently, however, the blood enzyme activity soars to very high levels following an acute myocardial infarction. In animal experiments, such high levels of enzyme activity have been recorded only in instances of very large infarcts.[3-6] Occasional patients have been cited with very high serum levels of glutamic oxalacetic transaminase activity prior to death from a myocardial infarction, and autopsy disclosed central necrosis of the liver.[7, 8] It has been postulated that the liver may contribute to these unusual levels of blood enzyme activity.[8, 9] There has not, however, been an extensive clinical survey of this problem.

From the Department of Medicine, The New York Hospital-Cornell Medical Center, New York, N. Y.

Recently we observed a young man with advanced rheumatic heart disease and tight aortic stenosis who developed progressive hepatic dysfunction (fig. 1) during his final illness and showed a rise in serum glutamic oxalacetic transaminase activity to 1,912 units (W.D., table 1). At postmortem examination there was severe acute central necrosis of the liver (fig. 2). Coincidentally there was a report of a similar patient with a very high transaminase activity in whom a clinical diagnosis of hepatitis had been considered although hepatic central necrosis was demonstrated at autopsy.[10] These observations stimulated an extensive review of our experience at The New York Hospital with patients having cardiac disease and high levels of serum glutamic oxalacetic transaminase activity, and this review forms the basis of the present report.

Materials and Methods

The records were reviewed of every patient who had a serum glutamic oxalacetic transaminase activity (SGOT) greater than 500 units per milliliter per minute as determined in the Liver Laboratory of The New York Hospital from January 1956 through July 1958. The records of those patients who had primary cardiovascular disease and high levels of enzyme activity were selected for special study. No patients with clinically evident viral hepatitis, toxic hepatitis, cirrhosis, or biliary tract disease were included.

The recorded clinical information was scrutinized for evidence of heart failure and hypotension. Right heart failure was diagnosed when the venous pressure was elevated and the liver was enlarged and tender. The level of venous pressure was measured by direct saline manometry and referred to the estimated level of the right atrium, or was estimated by inspection of the neck veins. Hypotension was diagnosed when the systolic blood pressure was 20 mm. Hg below the previously recorded levels on 2 or more consecutive bedside determinations. A diagnosis of clinical shock was made when specific comments indicated

he presence of severe hypotension, cold wet skin, nd peripheral cyanosis, usually accompanied by hanges in mentation.

In those cases in which an autopsy had been erformed, the sections of the liver and other issues were carefully reviewed. The microscopic ppearance of the hepatic sections was categorized s follows: normal, central congestion, central epatic cord atrophy, central fibrosis, acute central ecrosis. A careful attempt was made to distinguish etween chronic or long-standing and acute changes n the hepatic architecture. Central fibrosis or trophy was interpreted as evidence of chronic hange. Central atrophy was diagnosed when the plates or cords of hepatic cells radiating toward the hepatic vein were thin, often not reaching the central vein, without evidence of acute necrosis, and the sinuses were dilated. In evaluation of acute central necrosis the criteria described by Osserman and Ellenberg[11] were utilized: eosinophilic staining of the central area sharply contrasting with the basophilic stain of the normal liver cells; nuclear changes including pyknosis, fragmentation, occasional large pale nuclei, and occasional free nuclei; invasion by polymorphonuclear leukocytes; more or less architectural disruption depending on the age of the lesion. Central congestion, though often present, was not considered essential for a diagnosis of acute central necrosis.

Further to evaluate the clinical and autopsy correlations that became apparent among those patients with high transaminase activity, a second series was accumulated from the autopsy files of The New York Hospital. This group will be termed a "Control Series," since it was selected without regard for the level of SGOT activity. The records of all patients who died primarily from cardiac disease and were autopsied during the 30-month period from January 1956 to July 1958, and who had SGOT activity determined within 30 hours before death, were reviewed. Eighteen patients met these criteria, none of whom had clinical or autopsy evidence of primary hepatic disease, and constitute the "Control Series."

Serum transaminase activity was determined by a modification of the method of Karmen[12] and was reported as units per milliliter of serum per minute (hereafter abbreviated to units). Blood samples showing hemolysis were routinely discarded.

Results

During the 30-month period covered by this study, 12,566 glutamic oxalacetic transaminase determinations were performed upon 4,761 patients in the Liver Laboratory of The New York Hospital. In 157 patients, at least

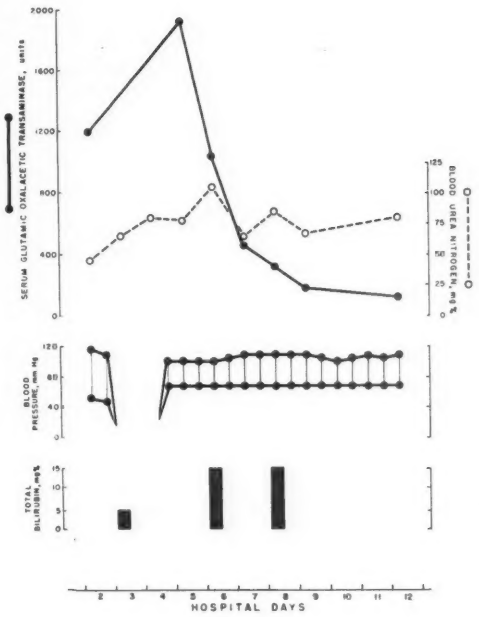

Figure 1

Clinical course of case 3. Hepatic and renal insufficiency accompanied progressive circulatory failure from multivalvular rheumatic heart disease.

1 determination was above 500 units; most of these had liver disease. In 17, however, the high SGOT levels occurred in patients suffering from cardiac disease without evidence of primary liver or biliary tract involvement (table 1). Eleven of these 17 patients were originally admitted because of an acute transmural myocardial infarction proved by the development of a Q-wave and RT-segment elevation in certain leads of the electrocardiogram. The 6 other patients were admitted for treatment of severe heart failure due to various causes. Ten patients died after the SGOT elevation was recorded; 5 of these had myocardial infarction and 5 had severe failure without recent myocardial infarction. Postmortem examinations were performed in 8 of these patients; 4 had myocardial infarction, 4 did not (table 2).

The maximum transaminase levels recorded in each patient ranged from 524 to 6,570 units. Five patients had peak levels of more

Table 1

Clinical Data in Seventeen Patients with Heart Disease and Serum Glutamic Oxalacetic Transaminase Activity >500 Units

Case	Clinical diagnosis	Right heart failure	Hypotension preceding SGOT determination >500 u. Severity	Hypotension preceding SGOT determination >500 u. Duration hours	Peak SGOT activity, units	Outcome
W.T.	HCVD, acute MI, CHF, aortic dissection	+++	+++	?	6570	Died, autopsy
D.O.	Acute MI	+++	+++	24	3850	Died, autopsy
W.D.	RHD, CHF	+++	+++	32	1912	Died, autopsy
H.S.	HCVD, arrhythmias, CHF	+++	+++	8	1588	Died, autopsy
C.L.	Acute MI	0	+	12	1256	Survived
I.R.	ASHD, previous MI, CHF	+	+	?	954	Died, autopsy
R.L.	Acute MI	+	+	24	870	Died suddenly 2 weeks later, autopsy
N.S.	HCVD, acute MI	+	+	14	760	Survived
A.T.	Acute MI	+	+++	8	736	Died, autopsy
A.G.	ASHD, CHF	+	+++	>8	734	Died, autopsy
J.C.	RHD, CHF	+++	+	6	653	Died
L.G.	Acute MI	+	+	>6	568	Survived
H.P.	RHD, CHF	+++	+	>6	554	Survived
E.K.	HCVD, acute MI	+	+++	>8	550	Survived
D.D.	Acute MI	+	+	>6	546	Survived
W.S.	Acute MI	+	+	?	526	Died
W.L.	Acute MI	+	+	8	524	Survived

Abbreviations: SGOT, serum glutamic oxalacetic transaminase; HCVD, hypertensive cardiovascular disease; MI, myocardial infarction; CHF, congestive heart failure; RHD, rheumatic heart disease; ASHD, arteriosclerotic heart disease; +, present, moderate; +++, present, severe.

than 1,200 units, and only 1 of these survived. Although the number of patients involved is small, table 1 indicates that the higher the SGOT in patients with cardiac disease, the worse is the prognosis. Five of 7 with SGOT between 500 and 700 units survived, whereas 8 of 10 with levels greater than 700 units died.

Serum transaminase activity usually fell sharply after the peak level and was often normal within a week in those patients who survived the initial episode (fig. 3). In W.T., in whom shock persisted for 10 days despite vigorous treatment, the transaminase activity fell from a peak of 6,250 units to 200-350 units during a 4-day period and remained at that level until death.

The clinical association between the peak serum transaminase activity and the level of blood pressure was striking. In all 17 patients, the elevation of SGOT to more than 500 units was preceded by a fall in blood pressure producing significant hypotension or overt clinical shock (table 1). The duration of the hypotension could not always be determined precisely from the available record, but in those patients from whom adequate data were available hypotension had been present for at least 6 hours prior to the SGOT elevation. Sixteen of the 17 patients also had right heart failure of varying severity. The hypotension, heart failure, and very high transaminase level occurred in different settings: as a direct complication of acute myocardial infarction, in association with severe myocardial failure, and secondary to a rapid arrhythmia in a patient with a diseased heart.

Liver-function tests were frequently abnormal when SGOT activity rose to high levels. Serum bilirubins were elevated in 7 of 8 patients in whom the determination was available. Four patients had excessive increases in prothrombin time (over 60 seconds by the

Quick 1-stage method) following the administration of usual doses of coumadin-derivative anticoagulants and required treatment with vitamin K. In 3 other patients anticoagulants were administered without difficulty. Five patients without anticoagulant therapy had prothrombin time determinations more than 2 seconds above the control. Serum alkaline phosphatase activity and flocculation tests were normal. Bromsulfaleinphthalein tests were not performed.

Pathology

Review of the protocols and histologic material from the 8 autopsied patients revealed 1 common pathologic finding—acute central necrosis of the liver. Four of the patients also had recent myocardial infarction, and 1 of these had a recent dissection of the aorta. Necrosis in other organs was not observed. Thus in 4 of the autopsied patients necrosis within the liver was the only source identified that could have been responsible for the elevation of the SGOT activity.

When the liver sections were graded according to the degree of central architectural collapse, loss of hepatic-cell outlines, and amount of liver-cell regeneration, the apparent age of the lesion correlated with the time from peak transaminase activity until death. In patients H.S., A.T., and D.O., in all of whom peak transaminase activity was recorded within 24 hours before death, the central liver cells were acutely necrotic but the cords extended to the central vein and the architecture was intact (fig. 4). In W.D. and W.T., in whom peak SGOT activity occurred 8 to 10 days prior to death, the central hepatic cells had disintegrated and the central stroma was compressed (fig. 2). The central spaces were filled with polymorphonuclear leukocytes, proteinaceous debris, red blood cells, and occasional free nuclei. There was also fibroblastic proliferation.

Patient R.L. was unusual in that the evidence for central necrosis was the regeneration of the cells around the central veins as indicated by large, pale, double nuclei with pale-staining cytoplasm. This observation, however, correlated with the clinical data (fig. 3) of a transient SGOT rise to high activity after an episode of hypotension followed by clinical recovery for 2 weeks until a sudden and unexpected death.

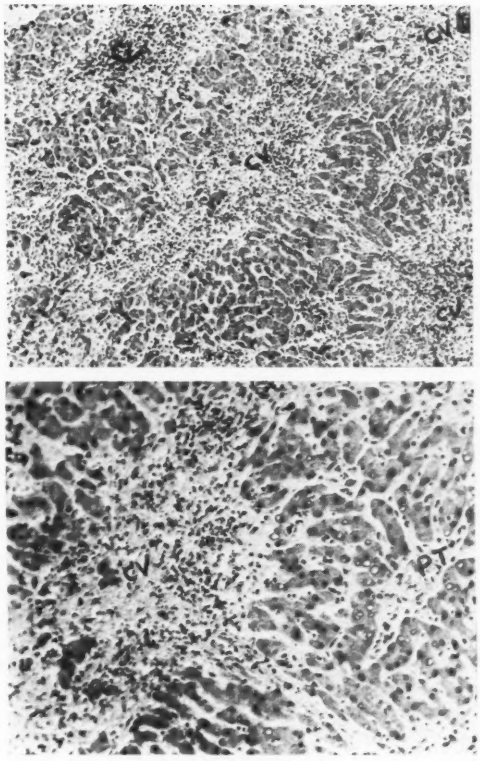

Figure 2

Top. *Section of liver from case 3 showing extensive necrosis of cells around central veins (CV). Note bridges of necrotic tissue connecting central areas.* Bottom. *Higher power of same section of liver. Area surrounding central vein contains islands of hepatic cells, round cells, polymorphonuclear leukocytes, proteinaceous debris. Note preservation of hepatic cells around portal triad* (PT). *Hematoxylin and eosin stain.*

In patients W.D., I.R., and L.G. there were central atrophy and some central fibrosis in addition to recent central necrosis in the liver. These findings correlated with the clinical data, since all 3 had long-standing cardiac disease with multiple episodes of right heart failure prior to their final admission.

Table 2

Autopsy Data in Eight Patients with Heart Disease Who Died after Serum Glutamic Oxalacetic Transaminase Activity Exceeded 500 Units

Patient	Time from peak SGOT to death	Autopsy findings	
		General	Liver
W.T.	10 days	ASHD, HCVD, recent MI, LVH, recent aortic dissection	Acute central necrosis
D.O.	24 hours	ASHD, HCVD, acute MI, LVH, intercapillary glomerulosclerosis, minimal microscopic pancreatic necrosis	Acute central necrosis
W.D.	8 days	RHD, LVH, RVH, aortic and mitral stenosis, mitral and tricuspid insufficiency	Acute central necrosis, central fibrosis
H.S.	24 hours	HCVD and ASHD with myocardial fibrosis, LVH, chronic cholecystitis, cholelithiasis	Acute central necrosis
I.R.	3 hours	ASHD, old MI, LVH, RVH	Acute central necrosis, central fibrosis
R.L.	15 days	Recent MI, ASHD, small renal infarcts	Recent central necrosis with regeneration
A.T.	24 hours	ASHD, acute MI	Acute central necrosis
A.G.	24 hours	ASHD, LVH, RVH	Acute central necrosis, central fibrosis

Abbreviations: ASHD, arteriosclerotic heart disease; HCVD, hypertensive cardiovascular disease; RHD, rheumatic heart disease; MI, myocardial infarction; LVH, left ventricular hypertrophy; RVH, right ventricular hypertrophy.

A few selected cases are presented to illustrate the clinical and pathologic correlations encountered in this group of patients with heart disease and high SGOT activity.

Case Reports

Case 1

D.O., a 62-year-old woman, known to have mild diabetes and hypotension, was admitted to The New York Hospital 6 hours after the onset of severe crushing precordial pain. Physical examination revealed hypotension, shock, a pulse of 20 per minute, pulmonary congestion, distended neck veins, edema, and an enlarged liver. The hematocrit value was 27 per cent, a white blood count 30,400 per mm.^{3} with 93 per cent polymorphonuclear leukocytes. A blood urea nitrogen was 108 mg. per cent. An electrocardiogram showed complete heart block and an acute posterior transmural myocardial infarction.

Twenty-four hours after admission SGOT activity was 3,850 units and a total bilirubin was 1.2 mg. per cent, with 1.0 mg. in the direct fraction. During the second day her blood pressure rose to 160/70 and the rhythm became normal at 80 per minute. Despite gradual improvement, she died on the third hospital day. Three hours before death, SGOT activity was 1,400 units.

Postmortem examination revealed a large, acute posterior infarction of the left ventricle extending into the intraventricular septum, right ventricle, and right atrium. There was an extensive acute central necrosis of the liver involving approximately 60 per cent of each lobule. Other findings were intercapillary glomerular sclerosis and patchy fat necrosis in the pancreas.

Comment

High SGOT activity in this patient was associated with a large myocardial infarction complicated by severe heart failure, complete heart block, and prolonged shock. Autopsy revealed in addition to the myocardial infarction an extensive central necrosis of the liver of recent origin. If the sections viewed under the microscope are representative a large proportion of the liver was necrotic. Although there was extensive destruction of heart muscle, leak of enzymes from the necrotic liver cells must have contributed in large part to the very high levels of serum transaminase activity recorded prior to death.

Case 2

A 47-year-old man, R.L., was admitted to The New York Hospital 6 hours after the onset of severe precordial pain. During the 18 months prior to admission he had occasional attacks of angina pectoris, and his physician had recorded blood pressures of 150 to 170/80 to 110.

On admission he had a blood pressure of 120/90 mm. Hg and a regular pulse of 100 per minute. There were basal rales, distended neck veins, and

an enlarged, tender liver. The venous pressure was 145 mm. of saline. An electrocardiogram showed a recent transmural anterior myocardial infarction.

SGOT activity 8 hours after the onset of chest pain was 77 units. Twelve hours after admission his blood pressure fell to 100/70 mm. Hg, without signs of clinical shock, remained low for 24 hours, and then gradually rose to 120/80. SGOT activity 8 hours after the onset of the hypotension had increased to 870 units (fig. 3). The patient improved until the sixteenth day after admission, when he suddenly had a recurrence of his chest pain and died.

Postmortem examination revealed a recent occlusion of the circumflex branch of the left coronary artery with an infarction of the anterior wall of the left ventricle estimated to be 2 weeks old. A mural thrombus was adherent to the infarcted area, and there were several small infarcts of the kidneys. Microscopic sections of the liver showed active regeneration of the hepatic cells around the central vein with large, pale, reduplicated nuclei.

Comment

High SGOT activity in this patient occurred after an acute myocardial infarction was complicated by heart failure and hypotension. The serum transaminase activity several hours after the fall in blood pressure was considerably higher than that usually encountered in patients with an uncomplicated myocardial infarction. The regeneration of liver cells around the central vein demonstrated at postmortem examination 2 weeks after the infarction is interpreted as evidence of an acute but limited hepatic central necrosis that was undergoing repair. The hepatic injury was probably secondary to the fall in blood pressure that occurred shortly after admission and was undoubtedly an important factor in the high level of SGOT activity that occurred at that time. The renal infarctions were quite small and most likely did not contribute significantly to the SGOT elevation.

Case 3

W.D., a 43-year-old man was admitted to The New York Hospital complaining of orthopnea and jaundice. Physical examination revealed deep jaundice and cyanosis with severe respiratory distress. Although he had profuse diaphoresis, cold skin, and an almost imperceptible pulse, his blood pressure was 120/95 mm. Hg. The cardiac rhythm

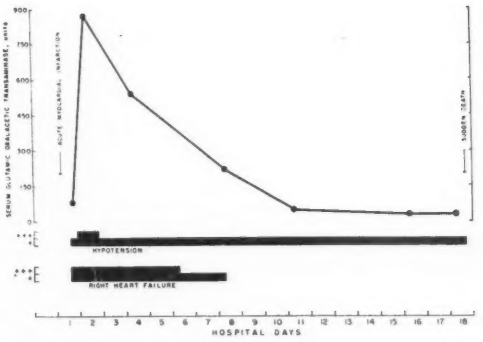

Figure 3

Clinical course of case 2. An acute myocardial infarction was complicated by right heart failure, hypotension, and high SGOT activity. Recovery was uneventful until sudden death on eighteenth day.

was atrial fibrillation at a rate of 90 per minute. He had physical findings of aortic stenosis and insufficiency, mitral stenosis and insufficiency, and tricuspid insufficiency. There were bilateral pleural effusions, rales over both lung fields, an enlarged tender liver, and ankle edema.

Shortly after admission SGOT activity was 1,206 units, a blood urea nitrogen was 45 mg. per cent, and a serum bilirubin was 4.9 mg. per cent (fig. 1). Two days later the blood pressure fell and became unobtainable for 32 hours, despite intermittent norepinephrine therapy. The rhythm then reverted spontaneously to normal at a slow rate and the blood pressure was maintained at 110/80 mm. Hg. On the fourth day the serum transaminase activity was 1,912 units, the serum bilirubin was 15.0 mg. per cent, with 9.2 mg. in the direct fraction, and the blood urea nitrogen was 105 mg. per cent. Five days after admission a venous blood ammonia was 190 gamma per cent (normal <100). He died on the thirteenth hospital day.

At autopsy the heart weighed 700 Gm. There was tight aortic stenosis, moderate mitral stenosis and insufficiency, and a widely dilated tricuspid valve. Both ventricles were hypertrophied. There was no evidence of necrosis in the myocardium or in any other organ but the liver. Sections of the liver demonstrated acute central necrosis involving about 40 per cent of the hepatic lobule engrafted on chronic central fibrosis and atrophy (cardiac cirrhosis) of moderate degree (fig. 2). The kidneys were hyperemic but otherwise unremarkable.

Comment

In this patient very high transaminase levels were associated with heart failure, shock,

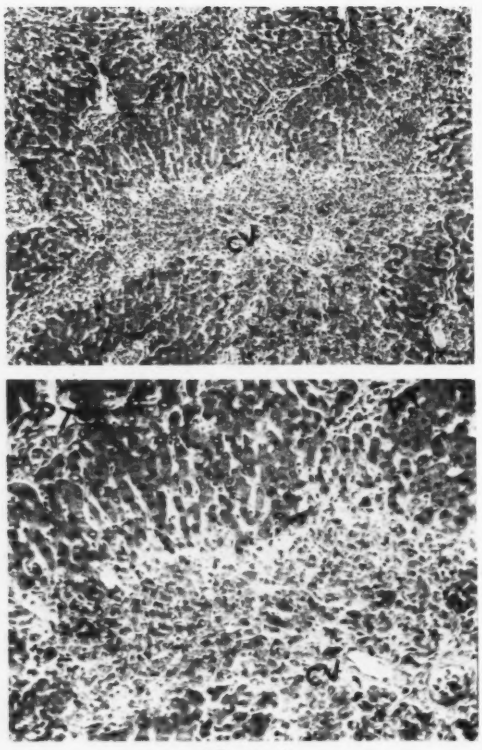

Figure 4

Top. *Section of liver from case 4 showing massive acute necrosis of hepatic cells surrounding central veins (CV) and bridges of necrotic tissue between central areas.* Bottom. *Higher power of same section of liver. Cellular outlines around central vein barely discernible. Invasion by round cells and polymorphonuclear leukocytes. Note intact cords near portal triad (PT). Hematoxylin and eosin stain.*

and progressive hepatic and renal insufficiency. The sharp reduction in hepatic blood flow associated with the shock produced liver failure, extensive hepatic central necrosis, very high SGOT activity, progressive bilirubinemia, and elevated blood ammonia. Although renal function also deteriorated secondary to the reduced blood flow, histologic changes in the kidneys were minimal. The only possible source found at autopsy for the great increase in SGOT was the recent necrosis of hepatic parenchymal cells around the central veins.

This case provides a striking illustration of the effect of progressive circulatory failure on liver function and structure. Several experienced clinical observers were of the opinion that the patient had developed a viral hepatitis. A case presenting similar clinical manifestations and diagnostic difficulties has been recently reported.[10] It has not been generally recognized that prolonged circulatory failure, manifested by hypotension, may lead to massive hepatic parenchymal destruction and liver failure.

Case 4

A 58-year-old man, H.S., was admitted to The New York Hospital, June 22, 1957, because of progressive dyspnea. His blood pressure was 160/94 mm. Hg, and the pulse was 130 per minute. There were basal rales, an enlarged tender liver, and peripheral edema. The day after admission a blood urea nitrogen was 15 mg. per cent and SGOT activity was 15 units. He developed sinus tachycardia with a rate between 135 and 150 per minute with frequent atrial premature contractions, then atrial fibrillation with a ventricular rate of 195 per minute and later atrial flutter (fig. 5). His blood pressure was unobtainable for 12 hours. Following quinidine therapy, normal sinus rhythm returned and his blood pressure rose to 130/70, but he died 2 days later. Twelve hours after the onset of hypotension his blood urea nitrogen had risen to 66 mg. per cent and SGOT activity was 1,588 units.

At autopsy the heart weighed 790 Gm. Both ventricles were dilated and hypertrophied. There were scattered small areas of myocardial fibrosis, but no coronary occlusion or myocardial infarction. Sections of the liver revealed extensive, acute central necrosis involving about 40 per cent of each hepatic lobule (fig. 4). The necrotic cells were sharply demarcated from the normal liver cells, but the over-all architectural pattern was well preserved without fibrosis, indicating recent injury. The kidneys were normal. There was also chronic cholecystitis and cholelithiasis without evidence of recent acute inflammation.

Comment

In this patient progressive myocardial failure and a very rapid ventricular rate were associated with low blood pressure and high SGOT activity. The hypotension was almost certainly a manifestation of a very low cardiac output with a concomitant reduction in hepatic perfusion. The central necrosis of the liver was the only source identified at post-

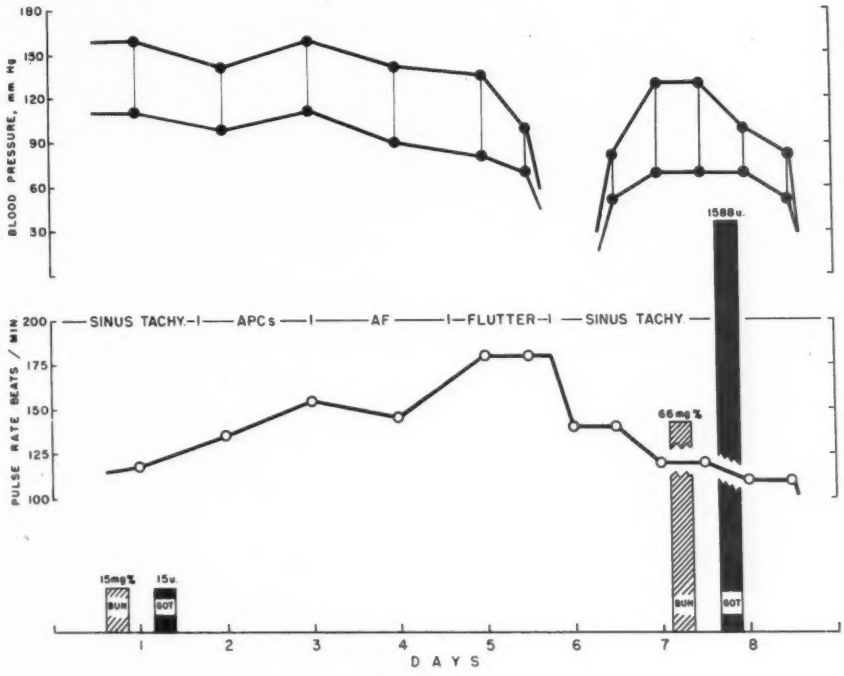

Figure 5

Clinical course of case 4. High SGOT activity and hepatic necrosis occurred in setting of heart failure, arrhythmias, and hypotension.

mortem examination for the increased SGOT prior to death.

Control Series. The clinical and autopsy data from the control series confirm and amplify the correlations noted in the group of patients with high transaminase activity. SGOT activity ranged from 17 to 408 units in the 18 patients of the control series.

Seven patients had normal SGOT activity prior to death (table 3). In none was central necrosis of the liver demonstrated at autopsy, and none had hypotension or shock prior to death. Four of these patients died during a bout of right heart failure, and in 3 the failure was severe. Noteworthy is case 3, who died from cor pulmonale associated with kyphoscoliosis, severe right heart failure (venous pressure greater than 300 mm. saline) and deep cyanosis. Despite these abnormalities the blood pressure was maintained and SGOT activity was normal 16 hours before death. At postmortem examination the liver showed central congestion without necrosis (fig. 6). In case 4, SGOT activity was normal 4 hours prior to death from advanced rheumatic heart disease with severe right and left heart failure without hypotension. Autopsy revealed a severely congested liver without evidence of acute necrosis (fig. 7). From this small group of patients it may be concluded that venous hypertension alone is probably not associated with either acute central necrosis of the liver or elevation of the SGOT activity.

The data from the 11 patients with elevated SGOT activity (table 3) provide further confirmation of the relationships noted previously in the group of patients with very high serum enzyme activity. All 5 of the patients with hepatic central necrosis had hypotension for more than 6 hours prior to death. In all but 2 of the patients with elevated SGOT ac-

Table 3

Control Series. *Clinical and Autopsy Data in Eighteen Patients Who Died of Heart Disease and Had Serum Glutamic Oxalacetic Transaminase Activity Determined within Thirty hours before Death*

SGOT level, units	Case number	Right heart failure	Hypotension		Acute central necrosis of liver	Myocardial necrosis
			Prior to SGOT	>6 hours prior to death		
Normal	1-7	3-6	—	—	—	—
46 - 100	8-12	8, 11, 12	8, 9, 10	8, 9, 10	8, 9, 10	10, 12
101 - 200	13-15	13-15	—	—	—	14, 15
201 - 500	16-18	18	16, 17, (18?)	16, 17, 18	16, 18	16, 17

tivity, the increased enzyme activity could be related to either myocardial necrosis or acute hepatic necrosis or both. Of the 2 without evident tissue necrosis at postmortem examination, 1, case 13, died of a saddle embolus. The other, case 11, died of rheumatic heart disease and failure. One patient, case 17, died after prolonged hypotension, but did not have central necrosis. Precise correlation of the level of SGOT activity with the hepatic architecture and the degree and duration of the hypotension was not possible because of the variation in the time blood samples were obtained for enzyme activity determinations.

Discussion

This study has demonstrated that a group of patients suffering from heart disease with very high levels of SGOT activity had several factors in common: right heart failure, hypotension or shock preceding the SGOT elevation, and, in those autopsied, central necrosis of the liver. All 8 patients who came to autopsy had acute central necrosis of the liver, which appeared to be related in time to the elevated serum transaminase activity. Clinical and laboratory data from the 9 patients who were not autopsied were essentially similar to those from the autopsied group, and it is reasonable to assume that they also had an acute central necrosis of the liver at the time of the high SGOT activity. In no patient was there clinical, laboratory, or pathologic evidence of either hepatitis or active biliary tract disease. In none could other causes of elevated transaminase such as pulmonary infarction, skeletal muscle injury, brain injury, acute pancreatitis or drugs[2] be incriminated.

In 11 patients the acute central necrosis of the liver was related to circulatory changes associated with an episode of acute myocardial infarction. Necrosis of both the heart and the liver undoubtedly contributed to the elevated SGOT activity in this group, but the level of activity was considerably higher than that usually associated with an uncomplicated myocardial infarction. In 6 patients, 4 of whom were autopsied, there was no evidence of infarction of the heart and the very high levels of transaminase activity were probably related to liver necrosis alone.

Although both Popper[13] and Sherlock[14] have emphasized the difficulties in interpreting postmortem sections from the liver, the acute hepatic necrosis encountered in this study is clearly a reflection of antemortem events. Acute central necrosis is not an invariable finding at autopsy, even in patients with severe heart disease,[11] and was not encountered in the 7 patients from the control series who had normal SGOT activity prior to death.

It may be concluded, then, that irrespective of whether or not recent myocardial infarction be present, very high levels of SGOT activity (>500 units) in patients with cardiovascular disease and circulatory failure who do not have primary liver disease are a reflection of an acute necrosis of hepatic cells surrounding the central veins. Presumably loss of hepatic cellular integrity results in leak of the intracellular enzymes into the peripheral circulation and very high serum activity. Since current technics for enzyme assay measure the activity of the enzyme system not the concentration, it cannot be stated with

certainty that an increase in blood enzyme activity means an increase in blood enzyme concentration. The possibility that the changes in blood enzyme activity are secondary to changes in inhibitors or activators cannot be ruled out completely.

The data from the control series, selected without regard for the level of serum enzyme activity, reinforce the observation that, in patients with heart disease, central necrosis of the liver is associated with elevated SGOT activity and hypotension. Venous hypertension alone was not associated with either increased SGOT activity or acute central necrosis of the liver in the absence of a fall in blood pressure. That prolonged hypotension does not invariably lead to hepatic necrosis or elevated SGOT is indicated by case 17 of the control series. We have too few observations in patients with prolonged hypotension and no right heart failure to draw firm conclusions. Melby et al. have reported moderate increases in SGOT activity in hypotension due to endotoxin,[15] a condition not usually accompanied by heart failure. Plasma GOT activity increases to very high levels in dogs subjected to hemorrhagic shock, and the liver is a major source of this increase.[16] Any form of peripheral circulatory failure, if severe enough, can probably produce deficient hepatic perfusion and hepatic central necrosis with increased SGOT activity.

Isolated instances of acute central necrosis of the liver in patients with high SGOT activity have been previously reported.[8, 9, 17, 18] Published protocols indicate that most patients with acute myocardial infarction and very high serum transaminase levels have been hypotensive. Chinsky et al.[8] presented a patient with high SGOT activity and central necrosis of the liver following a prolonged arrhythmia, and postulated that the hypotension associated with the arrhythmia produced the liver damage.

The pathogenesis of acute central necrosis of the liver has been the subject of speculation since F. B. Mallory first described the histologic details more than 50 years ago.[19–21] He

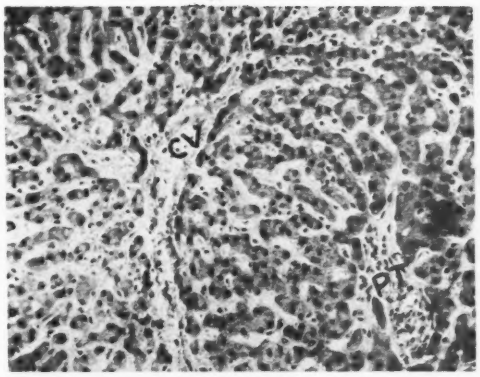

Figure 6

Section of liver from patient who died of cor pulmonale with severe venous hypertension, normal blood pressure, normal SGOT activity. Note intense congestion about central vein with thinning of hepatic cords and pyknotic nuclei. There is no evidence of acute hepatic necrosis. Control series, case 3. Hematoxylin and eosin stain.

was careful to differentiate this lesion from chronic passive congestion of the liver and interpreted the acute necrosis as due to an acute "toxic state" in the antemortem period. Many workers have held that acute necrosis is caused by acute congestion of the liver. Balton[22] and Zimmerman and Hillsman[23] produced central necrosis by complete or partial occlusion of the thoracic inferior vena cava. Although the histologic lesions in the liver were interpreted as secondary to acute congestion (greatly increased hepatic venous pressure), their experimental procedures must also have severely compromised hepatic blood flow and oxygen supply.

Experiences during World War II indicated that acute central necrosis of the liver could develop in previously healthy patients following prolonged shock.[24, 25] In a detailed study of central necrosis Lambert and Allison[26] had probably recognized this same factor when they stated that the "only constant etiological factor in cases of hemorrhagic necrosis is a severe circulatory disturbance." In a recent study of centrilobular necrosis following myocardial infarction, Clarke[27] found 8 of 9 patients with central necrosis had developed shock after the myocardial in-

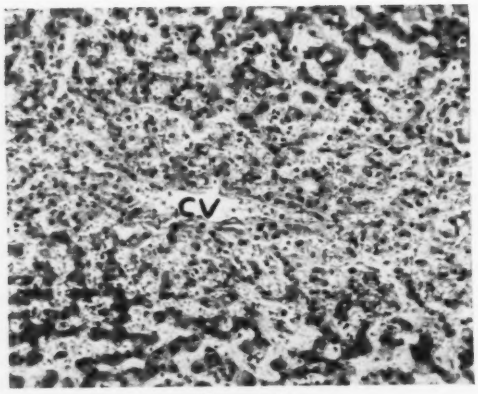

Figure 7

Section of liver from patient with severe right heart failure, normal blood pressure, normal SGOT 4 hours before death. Note intense central congestion and atrophy without acute necrosis. Control series, case 4. Hematoxylin and eosin stain.

faction. He concluded that the shock and not the frequently accompanying heart failure was the cause of the hepatic lesion. Ellenberg and Osserman[11] amplified and extended this opinion in a review of 200 unselected autopsies. Prolonged shock had been present in 32 of 34 patients with acute central necrosis. Noteworthy was the observation that severe congestive failure was not associated with acute central necrosis in the absence of shock.

Although every patient in the present series had a fall in blood pressure prior to the increased serum transaminase activity, only 6 developed overt clinical shock. That more did not have classic clinical shock is probably related to at least 2 factors. First, a diagnosis of shock was predicated on rigid criteria. Second, the absolute level of blood pressure is not an accurate measure of the absolute level of systemic or regional blood flow, and the latter are important in the production of acute central necrosis of the liver.

In patients with advanced heart disease and severe heart failure, a decline in blood pressure is generally an indication that cardiac output has fallen to such a low level that changes in peripheral vascular resistance are insufficient to maintain the previous levels of arterial pressure. Although it has been argued, particularly in reference to the hypotension of acute myocardial infarction, that the low blood pressure is largely the result of an inadequate adjustment of peripheral vascular resistance,[28] the weight of the evidence at the present time favors the view that an inadequate cardiac output is a primary fault. In patients with advanced aortic or mitral stenosis and reduced arterial pressures, data obtained at cardiac catheterization have shown a low cardiac output and high peripheral resistance.[29, 30] Rapid arrhythmias are frequently accompanied by a fall in cardiac output and arterial pressure.[31] Several studies have demonstrated that the hypotension of acute myocardial infarction is accompanied by a fall in cardiac output; resistance changes are variable.[32–34] It seems reasonable, then, to assume that the hypotension encountered in the 17 patients with very high transaminase activity reflected a significant drop in cardiac output.

It has been shown that hepatic blood flow parallels the level of cardiac output in patients with chronic heart failure and that the proportion of systemic flow diverted through the splanchnic bed remains essentially constant.[35, 36] Similarly, in burn shock[37] and hemorrhagic shock[38] hepatic blood flow declines in proportion to the changes in cardiac output. Cardiogenic shock must also be accompanied by severe restriction of hepatic blood flow and very low oxygen content in the hepatic veins. Hepatic and mesenteric oxygenation are maintained during shock by an increase in oxygen extraction per unit of flow producing a widened arteriovenous oxygen difference.[38, 39] Under such circumstances, those liver cells farthest from the arterial and portal supply and closest to the central veins are bathed in blood almost depleted of oxygen and other nutrients. At some critical point the vitality of the central cells is compromised and necrosis and loss of intracellular enzymes follow. If the circulatory reduction is prolonged, cells more and more distal to the central vein begin to deteriorate, producing a picture of massive necro-

is such as was encountered in W.T., D.O., V.D., and H.S.

In several cases with extensive central necrosis, there was a bridging of necrotic cells from one central area to another (fig. 4). If a patient should survive under such conditions, it is not difficult to visualize an extensive network of fibrous tissue eventually linking one hepatic lobule with another and resulting in a cardiac cirrhosis. The bridging between the central necrotic areas is completely in accord with Rappaport's experimental evidence that the terminal branches of the hepatic inflow circulation arborize not only around the central vein but also in the border area between the lobules equidistant from the hepatic triads.[40] These bridges of necrotic tissue, therefore, are further evidence of the role of circulatory changes in the pathogenesis of acute central necrosis.

Although all but 1 of the 17 patients with high SGOT activity had right heart failure, it is unlikely that venous hypertension alone produces either acute central necrosis or high levels of transaminase activity.[8, 41] We have studied 8 patients with proved chronic constrictive pericarditis and severe elevation of venous pressure; SGOT activity was normal in all. Four patients in the control series died from heart failure with severe venous hypertension, and although all had varying degrees of atrophy and fibrosis around the central vein, none had either acute central necrosis or elevated SGOT activity prior to death. The chronic reduction in oxygen tension in the terminal branches of the hepatic veins associated with right heart failure[42] may set the stage, however, for the rapid deterioration of the central cells after the development of cardiogenic shock and thus be synergistic with the acute reduction in hepatic blood flow.

The patients with high SGOT activity and myocardial infarction had extensive myocardial necrosis by electrocardiographic criteria and at autopsy. In the experimental animal the level of SGOT activity correlates well with the size of the infarct.[3-6] At least one of the experimental technics utilized to produce myocardial necrosis, however, is associated with prolonged cardiogenic shock.[3, 43] It is possible that in some experiments the high SGOT activity reflected both myocardial and hepatic necrosis. A correlation between the size of the myocardial infarction and the level of SGOT activity has not been established in man.

The association of high levels of transaminase activity and a poor prognosis in patients with cardiac disease has been noted by others, particularly with myocardial infarction.[7, 8] Since this study has demonstrated that a high level of SGOT activity in such patients implies hepatic necrosis, the correlation between serum enzyme activity and prognosis is probably not related strictly to the amount of myocardial tissue damage, but rather to the functional consequence of a given infarction. It is well known that myocardial infarction associated with shock carries a grave prognosis.[44] In terms of the function of the cardiovascular system, high levels of SGOT activity have the same clinical significance as shock and therefore signify a poor prognosis. Such high values, however, are more sensitive indicators of the level of hepatic perfusion than the blood pressure. This information may be useful at the bedside. Very high levels of SGOT in patients with cardiovascular disease should be an indication for vigorous attempts to treat the circulatory inadequacy whether or not overt clinical shock be present.

There is one final implication of this study that merits mention. Changes in blood enzyme activity are widely utilized to corroborate a diagnosis of myocardial infarction. Not infrequently if neither the clinical history nor the electrocardiogram is diagnostic, the interpretation of the changes in blood activity of SGOT or some other non-organ-specific enzyme such as lactic dehydrogenase may determine whether or not a patient is treated for acute myocardial infarction. If, as we have demonstrated, severe acute central necrosis of the liver produces striking elevation of serum enzyme activity, it is not unlikely that a minor degree of hepatic necrosis following

acute circulatory changes may result in lesser elevations and indeed produce an activity curve mimicking that seen in myocardial infarction. We have recently observed 2 elderly patients with complete heart block who had transaminase activity curves typical of myocardial infarction when their ventricular rate slowed and blood pressure fell for several hours. In one the bradycardia was secondary to high potassium and in the other to overdigitalization. In neither was there clinical or other laboratory evidence of acute myocardial infarction. The implication is clear: SGOT-activity changes similar to those of myocardial infarction may occur secondary to central necrosis of the liver unrelated to a myocardial infarct. Caution should be used in interpreting serum transaminase changes in patients with cardiac disease without diagnostic electrocardiographic or clinical abnormalities. Determination of glutamic pyruvic transaminase levels may be helpful in such situations, since elevation of the serum activity of this enzyme appears largely to reflect liver necrosis and is unusual in uncomplicated myocardial infarction.

Summary

A survey of all patients with very high serum glutamic oxalacetic transaminase activity encountered during a 30-month period at The New York Hospital revealed 17 patients with cardiac disease who had 1 or more serum glutamic oxalacetic transaminase (SGOT) determinations exceeding 500 units. None had clinical evidence of primary liver or gallbladder disease. Eleven were admitted with acute myocardial infarction, 6 with severe heart failure. All 17 developed hypotension or shock and all but 1 right heart failure prior to high SGOT activity.

Several patients had abnormalities of liver function when SGOT activity was very high. In 4 there was an excessive increase in prothrombin time following administration of anticoagulants.

In the 8 patients who came to autopsy there was histologic evidence of acute hepatic central necrosis. In 4 there was necrosis of both heart and liver, in 4 necrosis of liver alone.

Clinical and autopsy data from a control series of patients with heart disease selected without regard for the level of SGOT activity corroborate the association between hypotension, central necrosis of the liver, and increased SGOT activity. Increase in venous pressure in the absence of hypotension was not associated with acute central necrosis or elevated SGOT activity.

It is concluded that very high SGOT activity (>500 units) in patients with heart disease is at least in part caused by acute hepatic central necrosis secondary to a drop in cardiac output and reduced hepatic blood flow. Caution is urged in the interpretation of increased blood activity of intracellular enzyme systems as evidence for myocardial necrosis. Acute circulatory changes may result in hepatic necrosis and increased blood enzyme activity without myocardial infarction.

Acknowledgment

We wish to express our gratitude to Dr. Henry R. Erle for invaluable assistance during the collection of the transaminase data and to Dr. John T. Ellis, who reviewed the pathologic material.

Addendum

Since this manuscript was submitted for publication a similar study has been published, which also demonstrates a correlation between very high levels of SGOT and central hepatic necrosis in patients with heart disease. The authors concluded, however, that acute right heart failure was the precipitating cause of the liver necrosis. (Bang, N. U., Iversen, K., Jagt, T., and Tobiassen, G.: Serum glutamic-oxaloacetic transaminase activity as an index of centrilobular liver cell necrosis in cardiac and circulatory failure. Acta med. Scandinav. **164**: 385, 1959.)

Summario in Interlingua

Un revista de omne le patientes con elevatissime activitates de transaminase glutamico-oxaloacetic del sero, incontrate al Hospital New York in le curso de un periodo de 30 menses, resultava in un lista de 17 casos con morbo cardiac in que un o plure determinationes de transaminase glutamico-oxaloacetic del sero (TGOS) monstrava valores de plus que 500 unitates. Nulle patiente in iste serie exhibiva signo de un morbo primari del hepate o vesica biliari. Dece un habeva essite hospitalisate a causa de acute infarcimento myocardial, 6 a causa de sever discompensation cardiac. Omne le 17 disveloppava hypotension o cho e omnes, con un exception, habeva discompensation

dextero-cardiac ante le accusation del elevate activitate de TGOS.

Plure patientes habeva anormalitates del function hepatic quando le activitate de TGOS esseva multo elevate. In 4, un excessive augmento del tempore de prothrombina esseva notate post le administration de anticoagulantes.

In le 8 patientes presentate al necropsia, provas histologic de acute necrosis central del hepate esseva constatate. In 4 il habeva necrosis del hepate e del corde, in 4 solmente del hepate.

Datos clinic e necroptic ab un serie de controlo de patientes con morbo cardiac sed seligite sin reguardo al nivello del activitate de TGOS supporta le association inter hypotension, necrosis central del hepate, e augmento del activitate de TGOS. Augmento del tension venose in le absentia de hypotension non esseva associate con acute necrosis central o elevation del activitate de TGOS.

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Neuroses of the Heart

Angina Pectoris

Stenocardia, or the breast-pang described by Heberden, is not an independent affection, but a symptom associated with a number of morbid conditions of the heart and vessels, more particularly with sclerosis of the root of the aorta and changes in the coronary arteries. True angina, which is a rare disease, is characterized by paroxysms of agonizing pain in the region of the heart, extending into the arms and neck. In violent attacks there is a sensation of impending death.—William Osler, M.D. *The Principles and Practice of Medicine.* New York, D. Appleton & Company, 1893, p. 655.

Clinical and Cardiodynamic Effects of Adrenocortical Steroids in Congestive Heart Failure

By MURRAY A. GREENE, M.D., ARTHUR GORDON, M.D., AND ADOLPH J. BOLTAX, M.D.

THE TENDENCY of adrenocortical steroids and corticotrophin (ACTH) to promote salt and water retention, resulting in an increased extracellular fluid volume, has been established by many investigators.[1-5] Studies have also demonstrated the importance of excessive activity of the adrenal cortex in the pathogenesis of fluid retention and edema formation in various disease states, including heart failure.[6-11] In contrast, sodium and water diuresis and an improved response to mercurial diuretics, accompanied by clinical improvement, were reported in some patients with congestive heart failure during or following the administration of corticosteroids and ACTH.[12-19] The need to elucidate the problem of the effects of these agents in heart failure and the role of adrenal corticoids in the formation of edema are the basis for the present study of the effects of corticosteroids upon the clinical status, electrolyte balances, and cardiovascular dynamics in a group of subjects having heart disease of varied etiologies and congestive failure of varying severity.

Materials and Methods

Nine patients, 4 female and 5 male, with congestive heart failure are the subjects of this study (table 1). Ages ranged from 17 to 65 years. The patients are further classified according to etiology, functional capacity (New York Heart Association), and clinical status in table 1. Varied etiologies and mild to severe degrees of heart failure are represented.

The patients were hospitalized throughout the study and were semi-ambulatory or restricted to bed rest according to their functional capacities. A steady clinical state of at least 5 to 7 days' duration was required as a control period prior to physiologic testing. This state was maintained by the usual therapy for congestive heart failure and included a standard low-salt diet (less than 2 to 3 Gm. of sodium chloride daily). Mercurial diuretics were not given during this control period although most patients had received this therapy during the course of their previous illness.

During the control period, routine clinical observations were made and pertinent laboratory data obtained. Radioiodine uptakes by the thyroid gland were measured in order to evaluate the possibility that depression of thyroid function by steroids may secondarily affect clinical states and cardiodynamics. Phenolsulfonphthalein excretions and urea clearances were measured as gross indices of renal function. Fluid balances were evaluated according to daily fasting weights, daily urine outputs, and frequent determinations of serum and urine electrolytes.

Physiologic studies of cardiovascular hemodynamics by the standard technic of right heart catheterization were then performed (tables 2A and 2B). The patients were in the resting recumbent positions and were not given premedication. Duplicate measurements of cardiac output (direct Fick) were made. Pressures were obtained with the use of Statham strain-gage transducers (P23A) and recorded on a multichannel oscillographic photographic recorder. Mean pressures were obtained by electrical integration of the pressure pulses. Since flow and pressure measurements did not vary significantly during single studies, only average values are tabulated. Total pulmonary vascular, pulmonary "arteriolar," and total peripheral arterial resistances were calculated according to standard formulas (Poiseuille). Resistance is expressed as dynes sec. cm.$^{-5}$ by the use of conversion factors.* Total plasma volumes were determined by the I^{131}-labeled human serum albumin method and converted to total blood volumes by the use of peripheral hematocrit values. Predicted plasma volumes for either sex were based on the studies of Samet et al.[20] These values are essentially similar to those reported by other investigators. Pul-

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*1,332 = conversion factor from mm. Hg to dynes cm.$^{-5}$

Table 1

Clinical Status Prior to, during, and at Termination of Steroid Therapy

Patients	Steroid therapy	S*	Functional classification (N.Y. Heart Assn.)	Dyspnea†	Weight (lbs.)	Neck vein distention	Liver size (fingerbreadths below costal margin)	Peripheral edema‡	Pulmonary vascular congestion (x-ray)	Mercurial diuretic therapy
1. F., age 43 yr.	Prednisone									
CHF, etiology unknown	40 mg. 4 days	I	IV	Severe	132	90°	2	Marked	Moderate	None
	30 mg. 4 days	II	IV	Increased	139 (day 13)	90°	2	Increased	Moderate	None
	20 mg. 3 days	III	IV	Increased	138	90°	2	Increased	Moderate	None
	15 mg. 3 days									
2. F., age 37 yr.	Prednisone									
RHD: AS, MI, AI, MS	15 mg. 6 days	I	III	Moderate	91	90°	4	None	Moderate	None
	10 mg. 4 days	II	IV	Severe	97 (day 8)	90°	6	None	Moderate	2 ml. (days 8 and 10)
	5 mg. 3 days	III	III	Moderate	93	90°	6	None	Moderate	None
3. F., age 32 yr.	Prednisone									
RHD: MS	40 mg. 3 days	I	IV	Severe	93	90°	5	Marked	Moderate	None
	30 mg. 4 days	II	IV	Increased	97 (day 14)	90°	6	Increased	—	2 ml. (days 4, 9, and 13)
	10 mg. 8 days	III	IV	Severe	91	90°	5	Marked	Moderate	2 ml. + IV aminophyllin (day 14)
4. M., age 65 yr.	Triamcinolone									
IMH	16 mg. 5 days	I	III	Severe	164	90°	5	Marked	Moderate	None
	12 mg. 8 days	II	IV	Increased	170 (day 5)	Increased	6	Increased	Moderate	2 ml. (day 5), 2 ml. + IV aminophyllin (day 6)
		III	III	Severe	163	90°	5	Marked	Moderate	
5. M., age 41 yr.	Triamcinolone	I	III	Moderate	181	90°	4	Marked	Marked	None
RHD: MI, MS	16 mg. 14 days	II	IV	Severe	182 (day 10)	90°	4	Increased	—	2 ml. (day 10)
		III	III	Moderate	177	90°	4	Marked	Marked	None
6. F., age 17 yr.	Prednisone									
RHD: MS	40 mg. 5 days	I	II	Slight	104	None	1	None	Moderate	None
	30 mg. 4 days	II	II	Slight	106 (day 6)	None	1	None	Moderate	None
	15 mg. 5 days	III	II	Slight	105	None	1	None	Moderate	None
7. M., age 60 yr.	Prednisone	I	II	Slight	162	None	2	Slight	Moderate	None
HHD	15 mg. 13 days	II	II	Slight	160	None	2	Slight	Moderate	None
		III	II	Slight	160	None	2	Slight	Moderate	None
8. M., age 42 yr.	Triamcinolone	I	III	Moderate	134	90°	3	Slight	Marked	None
RHD: MS	16 mg. 13 days	II	III	Moderate	129 (day 13)	90°	3	Slight	Marked	None
		III	III	Moderate	129	90°	3	Slight	Marked	None
9. M., age 59 yr.	Prednisone	I	III	Moderate	140	45°	3	None	None	None
PF, E, CP	15 mg. 13 days	II	II	Slight	145 (day 10)	45°	3	None	None	None
		III	II	Slight	144	45°	3	None	None	None

For explanation of reference marks see opposite page.

onary vital capacities and maximum breathing apacities were obtained the day prior to catheterization with the modified Benedict-Roth spirometer.

After the control clinical and physiologic studies ere completed, adrenocortical steroids were administered for 13 to 16 days (table 1). Six patients received prednisone and 3 received triamcinolone.** These agents were selected because of their diminished tendency to cause soduim and water retention, edema, and potassium depletion as compared to other agents.[21–25] None of the other usual contraindications to steroid therapy was present.

Clinical status, routine laboratory data, and fluid balances were evaluated throughout the phase of steroid therapy. Radioiodine uptakes by the thyroid gland were measured prior to repeat cardiac catheterization. The previously stabilized cardiac regimen was continued during this period. Mercurial diuretics were given whenever warranted by the development of appreciable aggravation of clinical heart failure. Supplementary potassium therapy was not given.

Right heart catheterization and blood volume determinations were then performed on the last day of steroid administration; vital capacities and maximum breathing capacities were determined on the day prior to catheterization (tables 2A and 2B).

Clinical status and fluid balances were observed in some patients for several days following steroid withdrawal.

Changes in cardiovascular hemodynamics as a result of drug administration were evaluated in terms of statistical probabilities. In order to determine whether these changes may have been due to chance variation, or due to the action of an introduced variable (steroids in this study), 29 consecutive right heart catheterizations in this laboratory in adults with varied types of heart disease and with varying degrees of congestive failure were analyzed. In each of these studies at least 2 control "resting" cardiac output determinations (direct Fick), multiple pressure measurements, and resistance calculations were performed at intervals of 22 to 143 minutes (average 41 minutes), while the patients' states were considered to be constant. Chance or expected variations in terms of 95 per cent confidence limits (2 standard deviations from the mean variation) were established on this basis for the various measures of cardiovascular hemodynamics. A wide range of abnormalities in cardiodynamics was present in this control group, as would be expected in any series of patients with heart disease and congestive failure. It was therefore thought that greater reliability in an evaluation of the results would exist if these confidence limits were expressed in terms of percentage variations rather than changes in absolute values. Two exceptions are pulmonary "wedge" and right ventricular end-diastolic pressures. These parameters are expressed in terms of absolute changes because only minimal variations usually occur during a "resting" state and because small variations in their usually low numerical values frequently result in inordinately high percentage changes.

Changes in cardiovascular dynamics in the present series of 9 patients who received steroids were then evaluated in terms of statistical significance at the 5-per cent level. Changes that are considered to be statistically significant are appropriately indicated in tables 2A and 2B. It is appreciated that appraisals of the 2 sets of data in these subjects may not be strictly comparable with those of the control group because of the differences in time intervals. The possibility of greater variations in "resting" states may exist when studies are separated by greater time intervals. This factor does not appear to represent a major difficulty, however, since metabolic states could be studied and compared on the basis of oxygen consumptions and respiratory quotients.

Results

Three types of responses to steroid administration occurred in this series of patients:

**The authors wish to thank The Squibb Institute for Medical Research, New Jersey, for their supply of triamcinolone, (Kenacort ®).

*S. Time periods are denoted in this column. I, control observation period prior to initial physiologic studies; II, during the period of steroid administration (maximal changes and their time of occurrence are indicated); III, immediately prior to the repeat physiologic studies.

†Dyspnea. Severe if at rest, moderate if during mild effort, and slight if only during moderate effort.

‡Edema. Marked if involving more than lower extremities (presacral, abdominal wall), slight, if involving ankles.

AI, aortic insufficiency; AS, aortic stenosis; CHF, congestive heart failure; CP, cor pulmonale; E, emphysema; HHD, hypertensive heart disease; IMH, idiopathic myocardial hypertrophy; MI, mitral insufficiency; MS, mitral stenosis; PF, pulmonary fibrosis; RHD, rheumatic heart disease.

Table 2A

Cardiovascular and Pulmonary Dynamics before and at Termination of Steroid Therapy

Patient	S*	Heart rate (beats/min.)	Stroke volume (ml./beat)	A-V O_2 difference (ml.)	O_2 consumption (ml./min./M.²)	Respiratory quotient	Cardiac index (L./min./M.²)	Systemic artery O_2 (%)	Total blood volume (ml./ M.² BSA)	Total plasma volume (ml./ M.² BSA)	Predicted plasma volume (ml./ M.² BSA)
1	I	86	45	6.2	144	.76	2.37	84	3,690	2,440	1,460
	II	84	40	6.4	133	.76	2.08	73‡	3,610	2,310	1,460
2	I	87	33	7.0	155	.82	2.20	91	2,580	1,740	1,460
	II	92	27	8.5	152	.78	1.81	90	2,920	1,900	1,460
3	I	95	19	10.1	136	.78	1.35	89	3,780	2,290	1,460
	II†	—	—	—	—	—	—	—	3,370	1,850	1,460
4	I	110	38	5.2	110	.82	2.18	88	4,800	3,600	1,525
	II	126	22‡	6.2	94	.78	1.50‡	89	3,680	2,610	1,525
5	I	120	30	8.2	155	—	1.89	87	4,080	2,080	1,525
	II	110	32	9.5	177‡	—	1.85	93‡	3,680	1,840	1,525
6	I	74	50	4.7	124	.80	2.61	95	2,510	1,610	1,460
	II	68	50	5.3	127	.80	2.42	94	2,300	1,430	1,460
7	I	53	80	6.0	139	.80	2.33	88	3,710	2,150	1,525
	II	57	78	4.8	119	.84	2.47	91	2,870	1,490	1,525
8	I	76	36	7.2	115	.77	1.60	94	2,870	1,700	1,525
	II	70	40	7.0	119	.77	1.69	93	3,810	2,300	1,525
9	I	100	33	6.6	135	.84	2.04	82	3,030	1,210	1,525
	II	104	41	6.2	157‡	.84	2.60‡	82	3,210	1,380	1,525

*S. Physiologic studies before (I) and at termination of steroid therapy (II). Results tabulated are those during resting states.

†Unsuccessful.

‡Cardiovascular dynamic changes that are statistically significant (>2 standard deviations).

Table 2B

Cardiovascular and Pulmonary Dynamics before and at Termination of Steroid Therapy

Patient	S*	Pressure (mm. Hg)								Resistance (Dynes sec. cm.$^{-5}$)			Vital capacity (% predicted normal)	Maximum breathing capacity (% predicted normal)
		Peripheral artery			Pulmonary artery			Pulmonary "wedge" Mean	Right ventricular end-diastolic	Total systemic	Total pulmonary vascular	Pulmonary "arteriolar"		
		Systolic	Diastolic	Mean	Systolic	Diastolic	Mean							
1	I	169	85	110	75	29	47	—	18	2,250	900	—	53	46
	II	146	75	96	71	32	46	—	16	2,200	1,180	—	47	67
2	I	119	81	95	52	24	34	26	8	2,600	950	320	71	73
	II	131‡	102‡	114‡	63‡, §	—	—	—	16‡, **	3,820‡	—	—	66	76
3	I	94	66	75	69	40	59	47	21	3,200	2,600	800	40	37
	II†	—	—	—	—	—	—	—	—	—	—	—	37	37
4	I	138	82	100	—	—	—	—	19**	1,900	—	—	30	16
	II	120	80	94	53§	—	—	—	17**	2,600‡	—	—	30	15
5	I	149	98	117	71	49	55	26	19	2,580	1,200	660	78	72
	II	144	100	114	68	42	50	29	16	2,600	1,150	425	90	77
6	I	112	63	79	41	22	28	20	3	1,700	600	190	133	98
	II	120	69	86	41	19	24	16	1	1,950	570	210	133	98
7	I	230	103	137	65	25	36	19	11	2,600	700	295	72	58
	II	258	115	150	57	23	34	24‡	5‡	2,700	630	180‡	67	39
8	I	125	74	87	76	39	51	16	8	2,500	1,500	1,000	67	30
	II	131	76	90	83	36	55	—	9	2,500	1,500	—	64	31
9	I	118	89	99	60	30	40	3	7	2,350	840	850	64	20
	II	120	86	95	45‡	24‡	32	2	3‡	1,500‡	500‡	500‡	70	27

*See legend, table 2*A*.

†Unsuccessful.

‡Cardiovascular dynamic changes that are statistically significant (>2 standard deviations).

§ Right ventricular systolic pressure.

**Right atrial mean pressure.

Increases in congestive heart failure (5 patients), no significant changes in clinical states (3 patients), and improvement in clinical state and in cardiodynamics (1 patient).

Increases in Congestive Heart Failure

Clinical heart failure increased in severity in 5 (cases 1 to 5) of the 9 patients during steroid therapy (table 1). In 4 of these patients (cases 1 to 4) slight to moderate reductions in daily urine volumes occurred concomitant with increases in weight, indicating positive fluid balances. Laboratory data suggested worsening of hepatic function (increased bromsulfalein retention) and renal function (increased blood urea nitrogen and decreased urea clearance) in some patients of this group. All initially had normal 24-hour radioiodine uptakes (thyroid); no changes occurred during steroid therapy. Parenteral mercurial diuretics were given to 4 patients (cases 2 to 5) because it was considered hazardous to permit the deteriorated clinical conditions to persist. Responses to this therapy were poor.

Hyponatremia and hypochloremia existed in 2 patients in the control period, slight in one (case 1) and marked in the other (case 3). During steroid therapy slight depression of serum sodium occurred in 2 patients (cases 1 and 4) and of serum chloride in 2 (cases 1 and 3). In all 5 patients daily urine sodium and chloride excretions were markedly depressed in the control period. During steroid therapy excretion of these ions did not change or decreased slightly in 4 patients, whereas an increase occurred in 1 (case 2).

At the time of the second physiologic studies, 1 patient (case 1) who did not receive mercurials had a weight gain of 6 pounds and considerable increase in heart failure, and another (case 2) was somewhat improved by mercurial therapy but was still worse than his control state as well as 2 pounds heavier. The others had resumed their control clinical states after mercurial therapy although 2 (cases 3 and 5) were 2 and 4 pounds lighter.

In 1 patient (case 3) the second catheterization was unsuccessful. In the others very few statistically significant changes in cardiodynamics occurred (tables 2A and 2B). This lack of change could be attributed to the appreciable neutralization of the clinically obvious detrimental effects of steroids by mercurial therapy, which was administered until clinical improvement was obtained.

Total plasma volumes were elevated initially in all 5 patients, but they did not change much after steroid therapy. Slight falls in plasma volumes in 3 patients may well have resulted from mercurial diuretics used to combat congestive failure. No significant changes in vital capacities and maximum breathing capacities occurred as a result of steroid administration. In 3 patients (cases 1 to 3) in whom fluid balances were measured for several days following steroid withdrawal, no significant changes occurred in weights, urine volumes, and urinary excretions of electrolytes.

No Significant Changes in Clinical States

In 3 patients (cases 6 to 8) no changes in clinical heart failure occurred during steroid administration (table 1). A weight loss of 5 pounds was noted in 1 patient (case 8). Marked anorexia and diminished food intake as well as a decline in urine output and in urinary sodium and chloride excretions suggested that this weight loss was due to a decrease in tissue mass rather than to a diuretic response. No changes occurred in laboratory data or in 24-hour radioiodine uptake by the thyroid gland. In 2 patients (cases 6 and 7) control 24-hour urine sodium and chloride excretions were within normal limits; in the other patient excretions of these ions were diminished. Moderate reductions in daily sodium and chloride excretions occurred in these 3 patients during steroid administration.

No significant changes in cardiodynamics occurred as a result of corticosteroid administration except for a decrease in right ventricular end-diastolic pressure and pulmonary "arteriolar" resistance in 1 patient (case 7) (tables 2A and 2B).

Total plasma volumes were elevated initially in all 3 patients. In 2 (cases 6 and 7

slight to moderate decreases occurred during steroid therapy; in 1 (case 8) a moderate increase occurred.

No significant changes in vital capacities and maximum breathing capacities occurred as a result of steroid therapy. In 2 patients (cases 6 and 8) in whom fluid balances were studied for several days following steroid withdrawal, no changes occurred in body weights, urine volumes, and urine electrolyte excretions.

Improvement in Clinical State and in Cardiodynamics

In the patient (case 9) with pulmonary fibrosis and emphysema and cor pulmonale as the basic disease, there was an improvement in dyspnea during steroid therapy (table 1). However, a weight gain of 4 to 5 pounds and a slight decrease in daily urine output occurred concomitantly. There were no changes in routine laboratory data or in radioiodine uptake by the thyroid gland. A slight fall in serum chloride occurred.

Improvement in cardiodynamics resulted from administration of corticoids (tables 2A and 2B). There were a significant increase in cardiac output and lowered pulmonary artery systolic and mean pressures and right ventricular end-diastolic pressure. Although these changes in pressures may have been due to corresponding changes in intrathoracic pressure (which was not measured), the similarity of pulmonary "wedge" pressures in both studies suggests that the decreases in pulmonary artery and right ventricular pressures were due to decreases in effective intravascular pressures. Pulmonary vascular and pulmonary "arteriolar" resistances also decreased significantly. Oxygen consumption was higher during the second study and may have indicated some change in metabolic state. Nevertheless, the data indicate improvement in dynamics. Total plasma volume increased slightly with steroid therapy. Minimal, if any, improvement in vital capacity and maximum breathing capacity occurred.

No changes in fluid balance occurred following steroid withdrawal.

Discussion

The effects of ACTH and adrenal cortical steroids upon renal and cardiovascular systems in man and experimental animals have been far from uniform, so that generalization about the specific activities of these hormones is most difficult. The tendency of these hormones to promote salt and water retention and, consequently, an increase in extracellular fluid volume has been observed by many investigators.[1-5, 26] Corticoids that have prominent actions of this type include desoxycorticosterone, cortisone, hydrocortisone, and aldosterone. Newer synthetic derivatives have distinctly less tendency to cause salt and water retention although positive fluid balance has been observed, especially with higher dosages.[21-25] In contrast, some studies demonstrate that these hormones may possess diuretic properties. Davis and Howell[27] observed that both ACTH and cortisone produced an initial loss of salt and water in the intact dog. Other authors have reported natriuresis and chloruresis during the administration of ACTH in man[28] and in the rat.[29] Gaunt, Birnie, and Eversole[30] cited experimental data indicating that cortical hormones may produce diuresis in animals with normal water balance, in overhydrated ones, and even in mildly dehydrated ones.

In view of this diversity of steroid actions, it is not difficult to understand the uncertainties regarding clinical applicabilities of these hormones in patients with diseases characterized by positive fluid balances, particularly congestive heart failure. This difficulty is further compounded by studies that have demonstrated increased activity of endogenous salt-retaining adrenal cortical hormone, aldosterone, in these diseases.[7, 9-11, 31-33] In general corticoids have been used with caution in these conditions because of their tendency to promote salt and water retention. Various investigators, however, have reported favorable results following the administration of these hormones to patients with congestive heart failure.[12-19] Results, however, were not uniform in all studies. Some patients had

clinical and hemodynamic deterioration probably due to renal retention of salt and water.[12] In another study[18] a small number of patients demonstrated decreased urinary sodium excretion, increase in weight, and possibly increase in dyspnea.

The present study was undertaken to elucidate the effects of adrenal corticoids in congestive heart failure. In the 5 of the 9 patients in this series subjective and objective clinical manifestations and laboratory data indicated positive fluid balances with increased fluid accumulation and increased cardiac decompensation during the phase of steroid therapy. Responses to mercurial diuretics were poor. Heightened responsiveness during steroid therapy was not observed in any patient in this study. An evaluation of cardiodynamic changes is more difficult because of the effects of additional diuretic measures given to 4 patients during steroid therapy. These agents were given because of the development of precarious clinical conditions. It is therefore not surprising that no significant changes in cardiodynamics and some decreases in plasma volumes were noted in those patients given mercurial diuretics until clinical improvement occurred. In 1 patient (case 2) who was clinically worse and had an increase in plasma volume (in spite of 2 mercurial injections) at the time of the second cardiac catheterization significant increases occurred in pulmonary artery and right ventricular end-diastolic pressures.

In 3 of the remaining 4 patients no changes in clinical status occurred during steroid therapy. Some diminution in urinary sodium and chloride excretions occurred in these patients although the depression present in the first group was not observed. One patient (case 8) had a reduction in circulating volume and a decline in right ventricular end-diastolic pressure. A minimal increase in daily urinary volume in this patient would not appear to account completely for the diminution in circulating volume although it could have contributed to it. Another possibility may be that of internal fluid shifts.

In 1 patient (case 9) clinical symptoms improved during steroid therapy. This was the only subject who had pulmonary fibrosis and emphysema with cor pulmonale. This patient had improvement in cardiodynamics despite a positive fluid balance. It would be difficult in this case to define the primary mode of action of the steroid from the limited amount of data available concerning primary pulmonary functions. Possibilities include improvement in pulmonary inflammation, bronchomotor tone, and vascular resistance, causing a decline in right heart work load and, consequently, improvement in right heart function. Other workers have described favorable clinical results and improvements in pulmonary function following corticosteroid therapy in patients with chronic pulmonary disease.[34] This case is of interest in that clinical and hemodynamic improvement occurred only in that patient who did not have *uncomplicated* heart failure. Factors were probably present that are known to be favorably affected by corticosteroids, e.g., inflammation, and so forth. It is also probable that the additional fluid retained was distributed throughout the body compartments and any increase in fluid that might have occurred in the right heart was more than compensated by the favorable primary effect on the cardiopulmonary systems.

It is difficult to delineate those factors that may favor fluid retention during steroid therapy in some patients and not in others. The patients whose heart failure increased were those who generally seemed to have greater degrees of decompensation characterized by severe subjective manifestations, considerable fluid accumulation, and marked depressions in sodium and chloride excretions. Comparison of cardiovascular dynamics between the first 2 groups reveals much overlapping in many of the measurements. However, the generally higher pulmonary "wedge" and right ventricular end-diastolic pressures and total plasma volumes in those patients worsened by steroids suggest that deterioration is more likely to be produced in those patients who already have considerable fluid accumulation. The addition of agents that can retain

salt and water simply increases the severity of the existing positive fluid balance.

It is difficult to explain the differences in results in the present study from those reported by others, [12-19] in which diuresis and clinical improvement were noted in many patients with congestive heart failure. Evaluation of the effects of prolonged administration of various drugs in heart failure may be hazardous in view of the spontaneous variations that may occur in this state. Strict selection of patient material is also important. Subjects who are in a steady clinical state are best studied; those who have had acute deterioration in cardiac function prior to drug administration may not be satisfactory subjects for this way suggest variable extraneous adverse factors which by their presence or remittance may influence results. Concomitant therapy that may be detrimental to subjects with heart failure should be avoided. One group of investigators maintained high levels of water intake before and during steroid administration.[15-16] Leiter[35] has reviewed data which indicate that subjects with congestive failure may retain water excessively (resembling the effects of excessive antidiuretic hormone), leading to a worsening of clinical status. Steroids may have prevented overhydration in these patients[15, 16] as it does in water intoxication and as an antagonist to antidiuretic hormone[30, 36, 37] and may not have affected any of the originally existing abnormal cardiovascular-renal hemodynamics.

Patients were selected for the present study who were in a steady clinical state and who had heart disease of varied etiologies and of varying severity and no other condition that could be favorably affected by steroids, especially infectious diseases and overt inflammatory processes. The one exception was the patient with pulmonary fibrosis and emphysema. The results in this patient demonstrate the need for care in patient selection. The phase of drug administration was restricted to 13 to 16 days to minimize the possibility of spontaneous change in disease states.

Further work with agents that more specifically antagonize endogenous fluid-retaining factors may prove fruitful in patients with congestive heart failure. The prominent role of endogenous adrenocortical hormones (especially aldosterone) in retaining fluid and in promoting formation of edema in patients with heart failure has recently been emphasized. Drugs that may antagonize these hormones would be most important.[38]

Summary

This study is concerned with the effects of adrenocortical steroids in patients with congestive heart failure.

Nine adults with heart failure were studied during a steady clinical state (5 to 7 days), during administration for 13 to 16 days of prednisome (6 patients) or triamcinolone (3 patients) and in some subjects following steroid withdrawal. Daily determinations of fluid balances were made. Standard right heart catheterizations and measurements of blood volumes were performed prior to and at the termination of therapy.

Three types of responses to steroids occurred. In 5 patients increases in subjective and objective manifestations of heart failure and increased fluid retention occurred. Four of them developed precarious clinical conditions during steroid therapy, requiring mercurial diuretics, but with poor results. In general, cardiodynamic status at the termination of steroid therapy correlated well with clinical status. In 3 patients the clinical status did not change. Some depression in urinary sodium and chloride excretions were noted during therapy. Cardiodynamics were unaltered, except for an unexplained decrease in right ventricular end-diastolic pressure in 1 patient. In 1 patient clinical and cardiodynamic state improved, despite positive fluid balance. This was the only subject with primary lung disease (emphysema) and cor pulmonale.

These studies suggest that corticosteroids are generally detrimental in uncomplicated congestive heart failure. Greater deterioration appeared in subjects having the more severe degrees of decompensation, suggesting

that exogenous fluid-retaining influences were added to endogenous fluid-retaining forces, resulting in further accumulation of fluid in a circulatory system already burdened by hypervolemia.

Acknowledgment

The authors wish to express their appreciation to Miss Olga Gallego and Mrs. Josephine Damsky for their valuable assistance in the conduct of this study, and to Dr. Aaron S. Goldberg, Chemistry Department, The Bronx Hospital, for supplying the blood and urinary chemical analyses.

Summario in Interlingua

Iste studio es concernite con le effectos de steroides adrenocortical in patientes con congestive disfallimento cardiac.

Novem adultos con disfallimento cardiac esseva studiate in un stabile stato clinic (pro periodos de 5 a 7 dies), durante 13 a 16 dies de administration de prednisona (6 patientes) o triamcinolona (3 patientes), e in certe casos post le fin del curso de medication steroide. Esseva effectuate determinationes diurne del balancias de liquido. Catheterismo dextere standard e mesuration del volumine de sanguine esseva effectuate ante le initiation del therapia e post su termination.

Occurreva tres typos de responsa al administration del steroides. In 5 patientes il habeva subjective e objective augmentos del manifestationes de disfallimento cardiac e augmentos del retention de liquido. Quatro del cinque disveloppava precari conditiones clinic durante le therapia steroide. Illes requireva diureticos mercurial, sed le resultatos non esseva bon. A generalmente parlar, le stato cardiodynamic al termination del therapia steroide se monstrava ben correlationate con le stato clinic. In 3 patientes le stato clinic non se alterava. Un certe depression del excretion urinari de natrium e chloruro esseva notate durante le therapia. Le cardiodynamica remaneva inalterate, con le exception del occurrentia de un inexplicate reduction del tension dextero-ventricular termino-diastolic in 1 patiente. In 1 patiente le stato clinic e cardiodynamic se meliorava in despecto de un positive balancia de liquido. Iste patiente esseva le sol con primari morbo pulmonar (emphysema) e corde pulmonal.

Le hic reportate studios suggere que corticosteroides es generalmente detrimentose in non complicate casos de congestive disfallimento cardiac. In subjectos con plus sever grados de discompensation le deterioration esseva plus marcate. Isto pareva indicar que exogene influentias liquido-retentori esseva addite, con le resultato de un accumulation additional de liquido in un systema circulatori jam suffrente de hypervolemia.

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Surgical Correction of Coarctation of the Main Pulmonary Artery

By WENDELL B. THROWER, M.D., WALTER H. ABELMANN, M.D., AND DWIGHT E. HARKEN

ADVANCES in heart surgery, catheterization, and angiography have called attention to constrictions of the pulmonary artery and its branches. Recently Schumacker and Lurie[1] described a calcified stenotic zone at the bifurcation of the pulmonary artery in a 14-year-old girl. This was dilated with a Kelly clamp. No postoperative physiologic studies were reported. Sondergaard[2] reported 3 cases with similar stenosis at the bifurcation. Two of these were associated with the tetralogy of Fallot, while the third had an atrial septal defect and valvular pulmonic stenosis. Although Sondergaard discovered the vascular obstructions at operation, none was corrected.

Several unoperated cases have been reported. Arvidsson and associates[3] demonstrated by angiography what appeared to be multiple congenital stenoses of the peripheral pulmonary arteries in 4 patients. Coles and Walker[4] reported a case of bilateral constriction of the main branches of the pulmonary artery, which was diagnosed by catheterization and angiography. Eldridge, Selzer, and Hultgren[5] reported 5 patients with stenosis of a branch of the pulmonary artery demonstrated by catheterization.

We have recently encountered 2 cases of stenosis of the main pulmonary artery. Although Williams, Lange, and Hecht[6] point out that stenosis is probably not unusual, our first case may represent a rare form, as the constriction was in the middle third of the main pulmonary trunk. The constriction was successfully treated by open operation and as such appears to be the first case treated definitively and studied with preoperative and postoperative cardiac catheterization. Fortunately there was also cinematographic documentation of the operation.

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Dr. Abelmann is an Established Investigator, American Heart Association.

Case Reports

Case 1

E.T., an 18-year-old white college sophomore was first seen by us in April 1957. When 4 years old, he had been hospitalized for pneumonia. At the age of 8 he was thought to have rheumatic fever and was in another hospital for approximately 1 month. His family was then told for the first time that he had a murmur. The record indicates that it was heard as a systolic murmur along the left sternal border and was accompanied by a thrill.

He did well except for episodes of epistaxis until the age of 15, when he began to have occasional "dizzy spells" on exercise. During the 3 years prior to admission he noted slight dyspnea on vigorous exercise. On examination in April 1957 he was well developed, weighed 166 pounds, and was 66 inches tall. The heart was not enlarged to percussion and was in normal sinus rhythm. A systolic thrill was present anteriorly in the second left intercostal space. The pulmonic second sound was heard poorly and was split on inspiration. A grade-III harsh systolic murmur was heard widely at the base but loudest in the second left intercostal space along the sternal border; it was transmitted toward the apex and into the neck.

Electrocardiogram (fig. 1) showed delayed right ventricular conduction, and cardiac fluoroscopy and x-ray films (fig. 2) showed right ventricular enlargement. The pulmonary artery segment was not enlarged on fluoroscopy. Cardiac catheterization in September 1957 showed a mean systolic gradient of 32 mm. Hg across what was presumed

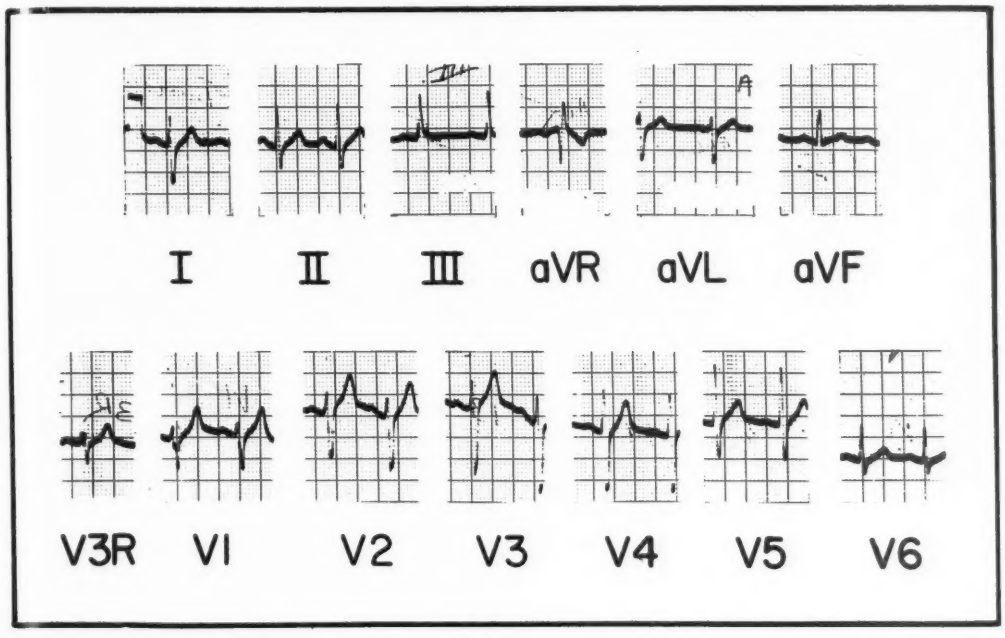

Figure 1

Case 1. Preoperative electrocardiogram showing delay in right ventricular conduction with pattern of incomplete right bundle-branch block.

to be the pulmonic valve (fig. 3*A*). The mean systolic pressure was 45 mm. Hg in the right ventricle and 13 mm. Hg in the pulmonary artery. The oxygen content of all right-sided cardiac chambers revealed no evidence of a left-to-right shunt. The arterial oxygen saturation was 94.5 per cent. A dilution curve with Evans blue dye from the right atrium to the brachial artery was normal. The cardiac index was normal at 3.3 L./min./M^2. A diagnosis of isolated moderate valvular pulmonic stenosis was made, and the pulmonic valve area was estimated as 1.0 cm.^{2}

Since the symptoms were minimal and the electrocardiogram and roentgenograms did not indicate severe right ventricular hypertrophy, surgery was deferred. Meanwhile, the boy worried about an impending operation, was unable to concentrate on his studies, did poorly in college, and surgery was requested sooner than had been elected initially.

On March 15, 1958, under hypothermia, through an anterior transverse bilateral incision in the fourth intercostal spaces, a stenosis or zone coarctation was found in the middle third of the main pulmonary artery (fig. 4). The diameter at this point measured 0.5 cm., whereas the proximal and distal portions of the main pulmonary trunk measured 2 cm. in diameter. Ideally one might elect excision of the zone of coarctation with end-to-end anastomosis. However, hypothermia would allow only a few minutes of circulatory arrest; therefore the stenotic area was incised longitudinally. This incision was extended proximally and distally. The valve was palpated and found to be normal. The lumen at the stenosis was less than 1 cm.^{2} in area due to a thickened fibrous wall remarkably like that seen commonly in coarctation of the aorta. The longitudinal incision was then closed transversely (fig. 5).

The postoperative course was uneventful except for right bundle-branch block that was first noted on the day after the operation. He was discharged on the fourteenth day after surgery.

Cardiac catheterization 6 weeks after surgery showed that the pressure gradient had been reduced from 32 to 9 mm. Hg (fig. 3*B*), at an identical cardiac index of 3.3 L./min./M^2. Repeated passage of the catheter across the obstruction under fluoroscopic observation demonstrated that the site of residual obstruction was about halfway between the pulmonic valve and the bifurcation of the pulmonary artery. The estimated area of constriction had been increased to 2.1 cm.,^{2} twice its preoperative size. Again blood samples

Figure 2

Case 1. Preoperative posteroanterior and right lateral chest roentgenograms suggesting right ventricular enlargement.

from the right-sided cardiac chambers showed no variation in oxygen content, and there was no evidence of other congenital lesions.

The patient is now asymptomatic. The second pulmonic sound is louder than preoperatively and the pulmonic systolic murmur less prominent. The electrocardiogram shows persistent right bundle-branch block.

Case 2

B. N., a 12-year-old white girl, was first seen in December 1957. At birth she appeared to be a normal baby. The family reported that since infancy, on exposure to cold her hands and feet had tended to become blue. Although she tired easily, she denied other symptoms. Because of peripheral cyanosis she had been seen at another clinic in 1954, when a heart murmur was noted and the family was told that the child possibly had a "small hole in the heart." During the year preceding our examination she had frequent episodes of epistaxis. When first seen by us, physical examination revealed a well developed, healthy appearing girl with strikingly red cheeks. The hands and feet were slightly moist and cool but there was no cyanosis, clubbing, or venous distention. The heart was not enlarged. The second pulmonic sound was louder than the second aortic sound, but not unusually loud or split. A grade-II basal systolic murmur was present, which radiated along the left sternal border and out toward the apex.

Electrocardiogram, cardiac fluoroscopy, and x-ray films were essentially normal. Right-sided heart catheterization in April 1958 showed a consistent fall in systolic pressure from the proximal to distal pulmonary artery, amounting to a mean systolic gradient of 6 mm. Hg. The site of transition appeared to be just proximal to the bifurcation of the pulmonary artery. There was no pressure gradient across the pulmonic valve. There was no evidence of coexistent cardiac lesions. Oxygen contents throughout the right side of the heart were identical. Arterial oxygen saturation was normal at 96.3 per cent. Indicator-dilution curves from the superior vena cava and the pulmonary artery to the brachial artery were normal. The cardiac index was rather high at 6.0 L./min./M.^{2} Thus a mild constriction of the pulmonary artery proximal to its bifurcation seemed to be defined. The hemodynamic disturbance did not require surgical correction.

Discussion

The pathogenesis of constrictions of the pulmonary artery has been debated.[2, 5, 7, 8] Most authors favor a congenital origin according to the Skodaic theory. This postulates a stricture produced by an extension of tissue from the ductus arteriosus into the pulmonary artery or aorta which contracts as the ductus undergoes obliteration. This explanation seems untenable in our first case since there was no

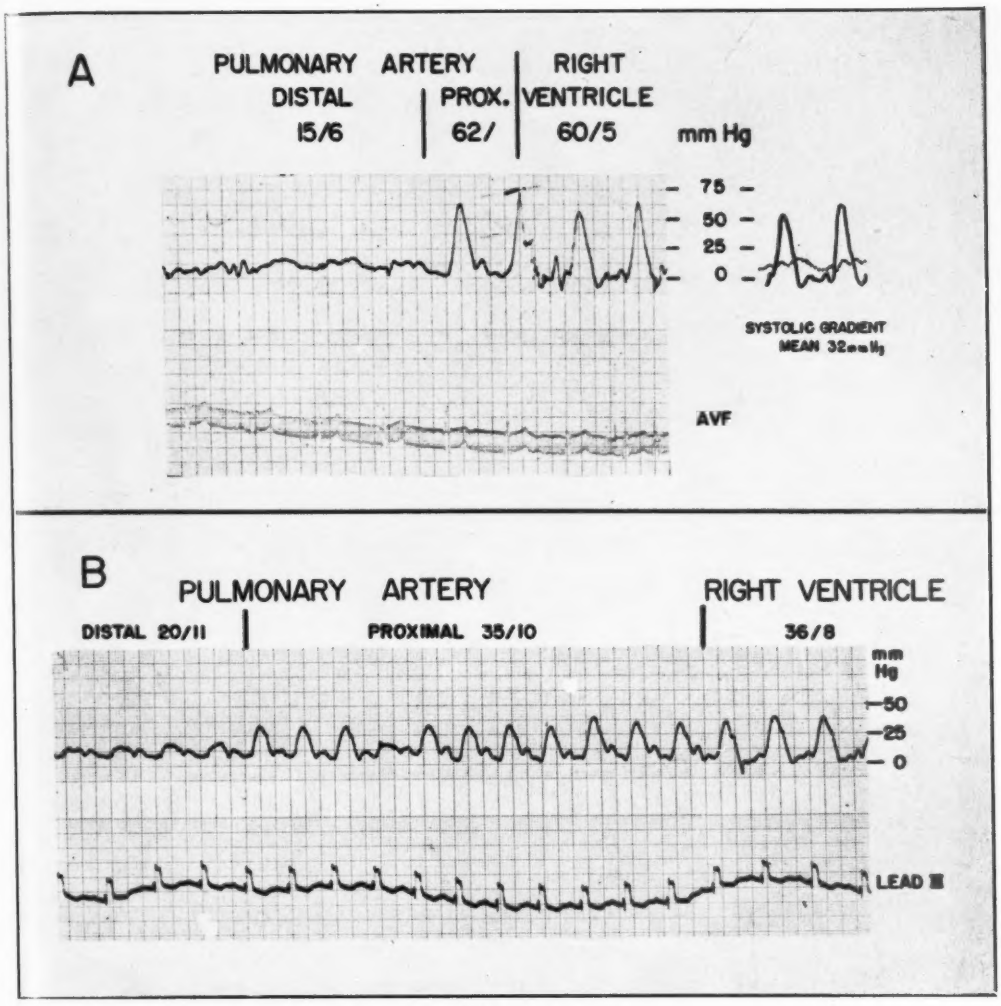

Figure 3

Case 1. Right heart pressures during preoperative (A) and postoperative (B) cardiac catheterization. Preoperatively (A) one pressure pulse was recorded from the pulmonary artery proximal to the region of obstruction. Although this is compatible with a diagnosis of coarctation of the pulmonary artery, this diagnosis was not made inasmuch as artifactual pressure tracings obtained from a catheter tip in a stenotic pulmonic valve may have the same appearance. The preoperative mean systolic pressure gradient of 32 mm. Hg (A) was reduced to 9 mm. Hg postoperatively (B). The cardiac output was the same during both studies: cardiac index = 3.3 L./min./M.² The estimated area of constriction was increased from 1.0 cm.² preoperatively to 2.1 cm.² postoperatively.

apparent connection between the ligamentum arteriosus and the stricture.

Attention has been directed to thrombosis of the pulmonary artery,[9-11] which at times has simulated pulmonary stenosis. We have recently operated on a patient for anomalous venous drainage associated with long-standing silent massive thrombosis of the pulmonary arteries. He succumbed when the anomaly was corrected at open-heart operation. It seems

Figure 4

Case 1. A view taken from the cinematographic documentation to show the zone of coarctation of the pulmonary artery.

likely that some of the cases diagnosed by cardiac catheterization and angiocardiography[3-5] but without anatomic clarification might represent partially resorbed occluding thrombi or emboli with recanalization. This is unlikely in our first case as the actual outside diameter of the pulmonary trunk at the coarctation was markedly narrowed and there was no evidence of thrombosis.

Thus none of the prevailing theories of pathogenesis seems to explain the isolated midzone coarctation of the main pulmonary artery of our first case. In the systemic circulation, the aorta may be narrowed at 2 sites and at times in the abdominal region.[12] Perhaps these are comparable vascular anomalies.

Postvalvular stenosis of the pulmonary artery is probably more common than is generally realized.[3, 4, 6] To our knowledge, however, pure stenosis, or what we prefer to call coarctation of the midportion of the main trunk of the pulmonary artery has not been reported.

The corrective technic in the first patient reported here had not been planned preoperatively. It is fortunate that hypothermia was being used. The possibility of unsuspected coarctation of the pulmonary artery may constitute an added indication for the routine use of hypothermia or mechanical bypass in what is *thought* preoperatively to be pure pulmonic valvular stenosis. In case 1 the stenosis was presumed to be critical on the basis of the preoperative pressure gradient of 32 mm. Hg and an estimated area of constriction of 1 cm.^{2} It has been emphasized by Holman[13] that when the diameter of the pulmonary artery is constricted to less than 50 per cent in animals heart failure results. A critical level undoubtedly existed in our patient with a diameter of 0.5 cm.

The diagnosis of anomalies of the pulmonary artery would be made more frequently if it were routinely considered in the differential diagnosis of pulmonary valvular stenosis. The absence of postvalvular dilatation, as in case 1, might make one consider coarctation of the pulmonary artery.

Catheterization studies should be helpful, but here again special care must be exercised to demonstrate the exact location of the stenosis or stenoses.

At the time of operation, proper exposure is mandatory in dealing with all congenital anomalies so that the unexpected may be detected.

Summary

Two cases of pure congenital coarctation of the main pulmonary artery are reported.

One case may be unique in that it represents the first report of an anatomically proved constriction located in the middle third of the main trunk. In this patient, successful open correction under hypothermia was carried out with cinematographic documentation. The results of preoperative and postoperative cardiac catheterization are described.

A plea is made for the routine use of open operation for correction of "pure pulmonic valvular stenosis." This allows better correction when the diagnosis is confirmed and flexibility for treatment if the unusual coarctation or constriction of the pulmonary artery is encountered.

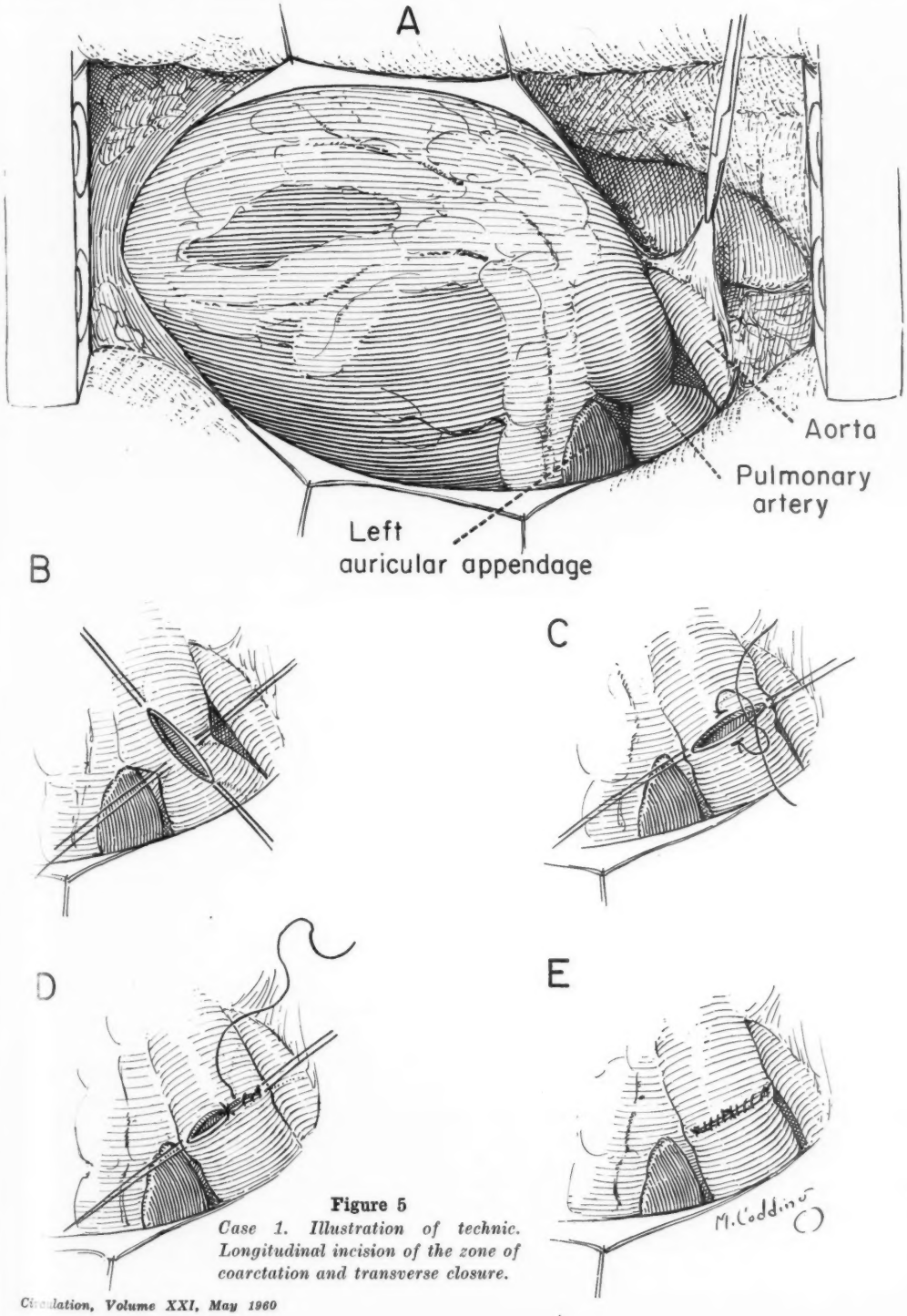

Figure 5

Case 1. Illustration of technic. Longitudinal incision of the zone of coarctation and transverse closure.

Summario in Interlingua

Es reportate duo casos de congenite pur coaretation del arteria pulmonar commun.

Un del duo es possibilemente unic in tanto que illo representa le prime reporto de un anatomicamente demonstrate constriction in le secunde tertio del trunco principal. In iste patiente, correction aperte esseva effectuate a bon successo sub hypothermia con documentation cinematographic. Es describite le resultatos de catheterismo cardiac ante e post le operation.

Es recommendate urgentemente le uso de chirurgia aperte in le correction de "pur stenosis pulmonovalvular." Iste technica permitte un correction superior quando le diagnose es confirmate; illo permitte un plus alte grado de flexibilitate in le tractamento in casos in que le phenomeno inusual de coaretation o constriction del arteria pulmonar es incontrate.

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Vesalius

The influence of Vesalius on the history of science may be regarded on the one hand in its general, on the other in its more special aspect.

Taking the general aspect first we may say that he founded modern anatomy. He insisted upon, and through his early unwearied labours by his conspicuous example he ensured the success of the new method of inquiry, the method of observation as against interpretation; he overthrew authority and raised up experience, he put the book of nature, the true book, in place of the book of Galen, and thus made free and open the paths of inquiry. . . .

Under a more special aspect he may be regarded as the founder of physiology as well as of anatomy in as much as he was the distinct forerunner of Harvey. For Harvey's great exposition of the circulation of the blood did, as we shall see, for physiology what Vesalius' *Fabrica* did for anatomy; it first rendered true progress possible. And Harvey's great work was the direct outcome of Vesalius' teaching, the direct outcome and yet one reached by successive steps, steps taken by men of the Italian school, of which Vesalius was the founder and father.—Sir M. Foster. *Lectures on the History of Physiology.* London, Cambridge University Press, 1901.

Epidemiology of Group-A β-Hemolytic Streptococci as Related to Acute Rheumatic Fever in Miami, Florida

A Six-Year Study

By MILTON S. SASLAW, M.D., AND JAMES M. JABLON, PH.D.

With the technical assistance of Madelyn Saul, B.S., and Sallie Anne Jenks

EPISODES of acute rheumatic fever are believed to be triggered by infections with group-A β-hemolytic streptococci, 10 to 35 days prior to the rheumatic complication. However, both rheumatic fever and rheumatic heart disease are infrequent in Miami, Florida.[1, 2] If the incidence rate of rheumatic disease depends on the frequency of group A streptococci, the low rheumatic rate in Miami should be accompanied by corresponding low streptococcal prevalence. A study was instituted in February 1953 to test this hypothesis.

Methods and Materials

Throat cultures were collected on swabs from 18,329 subjects between February 1953 and May 1958.[3–6] Each swabbing was carried out by holding 2 swabs together and passing the tips over the tonsillar areas and the posterior pharynx. One of each pair of swabs was plated on beef-infusion agar fortified with 4 per cent sterile defibrinated sheep blood; the other was plated on neopeptone-infusion agar fortified similarly. During the first 3 years, plates were prepared in our own laboratory; for the past 3 years, plates prepared commercially (Hyland Laboratories, Los Angeles, California) were used. After 24 hours' incubation at 37 C., plates were read for the presence of β-hemolytic colonies. Occasionally, poor growth necessitated an additional 24 hours' incubation, generally under anaerobic conditions. Colonies appearing to be β-hemolytic were subcultured until pure cultures were obtained. Verification of the nature of the organisms depended on β-hemolysis under anaerobic conditions, with identification by Gram staining after growth in Todd Hewitt broth. Pure cultures then were grouped according to the method of Lancefield,[7] by use of commercial (Difco) streptococcal antisera, groups A, B, C, F, and G.

During the same period of time, 4,265 blood samples were drawn, and antistreptolysin-O determinations performed on them, by the method of Rantz and Randall.[8]

Epidemiologic procedures for these studies varied from year to year, in order to provide answers to specific questions. Thus, a small group of children were followed repetitively for 3 years, from first through third grades;[5] a county-wide mass survey was conducted in children attending first through third grades;[5] variations in streptococcal incidence and prevalence as related to age were observed;[9] possibilities of streptococcal transmission from child to mother or mother to child were investigated;[10] streptococcal findings and illness resulting in school absence were studied;[10] a case registry of rheumatic fever and glomerulonephritis with intensive follow-up was instituted.[11, 12]

Results

Subjects were considered in the following *age* classes: 0 to 6, 6 to 9, 9 to 15, University students, adults, and mothers of second-grade children. The prevalence of β-hemolytic streptococci in these groups is presented in table 1. The findings indicate that the highest incidence was observed in the 6- to 9-year-old children (11.09 per cent), while the lowest was in the mothers of second-grade children (0.2 per cent). This low figure was obtained at the same time that 10.8 per cent of the second-grade children themselves harbored group-A β-hemolytic streptococci. These differences are highly significant ($p<0.0005$).

Throat swabs collected *monthly* for 8 consecutive months during the school year from a group of 6- to 9-year-old children yielded positive cultures for group-A β-hemolytic streptococci at least once in 30 to 40 per cent of the children studied (average 35.49 per cent).[3–6]

From the National Children's Cardiac Hospital, Miami, Fla.

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Table 1

Number and Per Cent of Group-A β-Hemolytic Streptococcal Isolates, According to Age of Subjects, Miami, Florida (January 1953 through June 1958)

Age of subjects	No. cultures taken	Group-A no.	β-hem. strep. per cent	All other β-hem. strep.*	No. of samples	Antistreptolysin-O titer average
0-6	764	21	2.8	47	0	—
6-9	11,248	1,247	11.09	2,013	1,808	96
9-15	2,091	151	7.2	394	0	—
18-23	631	7	1.0	63	631	96
23+ (adults)	1,812	40	2.2	195	1,611	73
Mothers of 2nd grade children	863	2	0.2	49	215	97
Epidemic=4/58	920	104	11.3	141	0	—
Repeats (A only)	100	64	64.0	64	0	—
Total	18,429	1,636	8.87	2,966	4,265	87

*Groups B, C, F, G and nongroupable strains.

Throat cultures taken *simultaneously* from mothers and children on 863 occasions (36 pairs of participants) yielded β-hemolytic streptococci from one or both subjects 169 times. The same group of β-hemolytic streptococci was recovered from the throats of both 5 times, and only once were group-A β-hemolytic streptococci found in the cultures from both parent and child.[10]

Antistreptolysin-O titers performed on blood samples drawn from children 6- to 9-years-old averaged from 82 to 103 units; University students 96 units; adults 73 units; mothers of second-grade students, 97 units.[4-6] All bloods were drawn when subjects were clinically well, except for 340 samples taken from children 7- to 8-years of age, among which were studies carried out during 78 respiratory absences. The average antistreptolysin-O titer of these 340 bloods was 82.

Daily absentee records were maintained on 53 second-grade students.[10] Cause of absence was determined each time. Thirty-four children had 78 respiratory illnesses (table 2). Throat cultures taken within 24 to 48 hours of initial absence, and prior to administration of antibiotics (which were given only rarely), were negative for β-hemolytic streptococci from 22 subjects, accounting for 41 absences attributed to respiratory infection. Data were not available in 1 episode. Streptococci were recovered from the throats of the remaining 12 children. Four children had 15 respiratory absences, during 10 of which group-C strains were isolated; 2 had 4 illnesses during 2 of which nongroupable strains were recovered. Ten group-A strains were recovered from the throats of the remaining 6 children who suffered 17 absences due to respiratory disease. Of these 6 children, however, only 2 developed their respiratory illnesses within 24 hours of the first isolation of the group-A organisms. In the remaining subjects, group-A organisms were isolated repeatedly for 7 weeks before illness in 1 subject, 6 weeks in another, 10 days in a third, and 7 days in a fourth. Two of the 6 children showed a rise in the antistreptolysin-O titer, only 1 of whom had an illness and initial isolation associated.

Epidemics

In February and March 1955, the appearance of increased numbers of group-A type-6 organisms* led to intensive study in 2 classrooms of 1 school.[6] This type of streptococcus was recovered from the throats of 46 per cent and 30 per cent respectively of those cultured. There was no indication in any other classes that these bacteria were prevalent. All but 1 of the 18 children with type-6 strains, from

*Typing was carried out by Dr. Elaine Updyke, of the Communicable Disease Center, U.S.P.H.S., Chamblee, Georgia, or with serum provided by her.

Table 2

Recovery of β-Hemolytic Streptococci from Throats of Thirty-Four Second-Grade Children during Seventy-Eight Respiratory Illnesses (October 1956-May 1957)

Total number of second-grade students studied				53
Respiratory illnesses:	children		34	
	illnesses		78	
No. without recovery of β-hemolytic streptococci:	children		22	
	illnesses		41*	
No. with recovery of β-hemolytic streptococci:	children		12	
	illnesses		36	
No. positive, group-C β-hemolytic streptococci:	children	4		
	illnesses with organism recovered	10		
Total illnesses		15		
No. positive, group-NG β-hemolytic streptococci:	children	2		
	illnesses with organism recovered	2		
Total illnesses		4		
No. positive, group-A β-hemolytic streptococci:	children	6		
	illnesses with organism recovered	10		
Total illnesses		17		

*Data not available in 1 episode of illness.

whom sufficient blood samples were available, showed a rise in antistreptolysin-O titer of 2 tubes or greater in serial studies. Despite these rises in titers, the number of absences did not increase. Absenteeism in these 2 classes was no different from that elsewhere in the school. Comparison with absence rates during other months, and with other schools, failed to show any evidence of a "clinical epidemic."

Early in April 1958, group-A type-12 organisms appeared on the increase in 1 school. Nine hundred children, the entire school population (except for 48 who refused or were out of town), had their throats cultured within 2 days. Those who were absent were reached at their homes. Group-A streptococci were isolated from 103 cultures (11 per cent), varying from 7 per cent among sixth grades to 16 per cent in the second grade. These findings are consistent with the average recovery rates previously mentioned as related to the age of children. However, data were collected by individual classes. Of the 29 rooms in the school not 1 had a group of children completely free of group-A organisms.* The range was from 3 per cent (1 of 34) up to 32 per cent (11 of 34). Five of the 7 classrooms with highest percentages (over 15 per cent) were on the first floor of the 2-story building, all emptying on to 1 portion of 1 corridor. One class with a high rate was on the second floor, at the head of the stairway leading to the same portion of the same corridor. The seventh class met on the ground floor in the school cafeteria. No correlations were evident between the streptococcal rates and absenteeism. Of the cultures taken in school, 11.2 per cent (96 of 856) were positive, whereas 17 per cent (7 of 42) of the absentees cultured were positive for group-A streptococci. This difference is not statistically significant ($p>0.20$). When there were siblings, the recovery rate paralleled the over-all school rates.

The case register[11, 12] of active rheumatic episodes maintained from October 1, 1955, through September 30, 1958, revealed the oc-

*A shortage of typing serum precluded the possibility of typing all isolated organisms.

Table 3

Episodes of Acute Rheumatic Fever in Dade County, Florida, as Reported by Practicing Physicians: Case Register Maintained from October 1955 through September 1958

Age of subjects	10/55-9/56		10/56-9/57		10/57-9/58		Total	
	Present*	Absent†	Present	Absent	Present	Absent	Present	Absent
0-2	0	1	0	0	0	0	0	1
3-5	0	1	0	0	1	0	1	1
6-9	3	0	5	4	3	0	11	4
10-12	1	1	6	4	5	0	12	5
13-15	1	0	3	3	3	0	7	3
15+	4	4	10	8	6	2	20	14
Unlisted	1	0	0	1	1	1	2	2
Subtotals	10	7	24	20	19	3	53	30
Totals	17		44		22		83	

*Present = Jones criteria for Rheumatic Fever satisfied.
†Absent = Jones criteria for Rheumatic Fever not satisfied.

currence of 83 episodes, of which 53 satisfied the modified Jones criteria (table 3). One quarter of the total were observed in children under 9 years of age; an additional third, in the 10- to 15-year-old group; 23 in subjects under 30 years old, and the remainder, in patients over 30.

Discussion

Obvious rheumatic fever is seen infrequently in Dade County, Florida,[12] as evidenced by the report of only 83 episodes in a 3-year period in a population averaging between 750,000 and 850,000. Group-A β-hemolytic streptococci, on the other hand, are recovered frequently from the throats of local school children, particularly in the 6- to 9-year-old bracket.[3-6] The high streptococcal incidence and prevalence findings do not correlate with the low rheumatic fever rate.

The infrequency of rheumatic illness might be attributed to the presence of unrecognizable forms of the disease. However, previous studies in Dade County showed that rheumatic heart disease also is infrequent in native-born Miami school children,[1] and that rheumatic heart disease is observed infrequently in autopsies performed on native-born deceased Floridians.[2]

Certainly the small number of rheumatic episodes cannot be attributed to a dearth of group-A β-hemolytic streptococci. Undoubtedly, other factors—climate, socioeconomic status, environment, and heredity, to name a few—must affect the mechanism whereby these bacteria exert their etiologic effect in leading to rheumatic fever.

The ubiquity of β-hemolytic streptococci and the high frequency of recovery from throat cultures suggest that these findings reflect the "carrier state" rather than infections.[5] If infection is defined in terms of clinical illness, undoubtedly the bulk of our isolations were from the throats of carriers. However, if subclinical infection is recognized as that condition in which bacterial recovery is accompanied by immunologic responses such as a rise in antistreptolysin-O titer, approximately 50 per cent of our positive children in serial studies showed a rise of 2 tubes or greater. Although this figure is lower than the 70 to 80 per cent reported from northern centers,[13] the difference is inadequate to explain the low-incidence rate of rheumatic fever.

What about clinical infection related to streptococcal recovery? In a study of school absenteeism due to respiratory illness, the streptococcal recovery rate was significantly higher than the rate of isolation from the throats of children who were not ill.[14] In a second study,[10] 6 of 78 illnesses attributed to respiratory causes were associated with the recovery of group-A β-hemolytic streptococci. In only 2 of these 6 children did the respiratory illness develop within 24 hours of the

initial recovery of a group-A organism; one had a rise in antistreptolysin-O titer. Mothers of the children in this portion of the investigation had their throats cultured at the same time as the children. No cross infection was apparent.

Although the group-A β-hemolytic streptococcus may be the cause of clinical illness (as seen by the higher recovery rate of these organisms from the throats of children ill with respiratory infections than from those not ill), the frequency of streptococcal isolation from the throats of well children, the failure to develop rises in antistreptolysin-O titer in respiratory illnesses occurring at the same time that group-A strains were isolated, and the observation that respiratory infections may be due often to organisms belonging to groups B, C, or G, confuse the streptococcus-illness situation in Dade County, Florida. This lack of clear-cut relationship between recovery of organisms and respiratory illness may be different in Miami from other climates, and in order to clarify the relationship between streptococcal findings, clinical respiratory involvement, and the incidence of acute rheumatic fever comparative studies in other geographic locations are essential.

Summary

Rheumatic fever and rheumatic heart disease are infrequent in Dade County, Florida.

Conversely, β-hemolytic streptococci are recovered frequently, particularly from the throats of children 6 to 9 years old.

The relationship between group-A β-hemolytic streptococci, clinical illness, and rheumatic illness may be different in South Florida from elsewhere in the United States.

Summario in Interlingua

Febre rheumatic e rheumatic morbo cardiac non es frequente in le Contato Dade in Florida.

Del altere latere, streptococcos beta-hemolytic es isolate frequentemente, super toto ab le gurgites de juveniles de inter 6 e 9 annos de etate.

Il es possibile que le relation inter streptococcos beta-hemolytic del Gruppo A, morbo clinic, e morbo rheumatic non es le mesme in Sud-Florida como altembi in le Statos Unite.

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Environmental Factors in Coronary Heart Disease

An Epidemiologic Study at Agra (India)

By KRISHNA S. MATHUR, M.D., F.R.C.P.

CORONARY heart disease, emerging from obscurity in the early years of the century, has become the most serious and immediate health problem in many countries. In New England the incidence has risen from 37 per cent of 2,314 cardiac patients in 1928[1] to 48.5 per cent among 3,000 heart patients in 1951.[2] A review of a series of 1,284 consecutive hearts examined by the injection-plus-dissection technic of Schlesinger, at the Beth Israel Hospital, Boston, during the decade 1941 to 1950 revealed myocardial infarction on gross and histologic examination of the myocardium in 268 or 20.6 per cent and occlusion of coronary arteries in 342 or 26.6 per cent of the hearts.[3]

Comparable statistics on the subject are not available in India but the disease has apparently assumed menacing proportions and can be presumed to be on the increase. Sanjivi,[4] Vakil,[5] Samani,[6] and Padmavati[7] have reported incidences of 11.8 to 13.5 per cent in their hospital series of cardiac cases. In a similar study of heart cases in private practice Vakil[8] found the frequency to be 26 per cent. Recently Malhotra[9] reported an incidence of 28.3 per cent in combined hospital and private series.

A most striking and potentially important fact about this disease is its geographic variability, i.e., the great differences in its incidence and mortality in different populations. It has been reported, for example, that middle-aged men in the United States, Great Britain, and Finland are more prone to die of coronary heart disease than are men of the same age in Norway or Denmark, and are several times more prone than are men in Italy or Portugal; the disparity is even greater when comparison is made with middle-aged men in Japan, with the Bantu in South Africa, the Mayan Indians in Guatemala, the Navajo in New Mexico, the Jews in Yeman, the natives in Okinawa, the Negroes in Nigeria, and so on. Gordon[10] observed that the death rate from arteriosclerotic heart disease is lower among Japanese in the United States than among white American men of the same age group, and it is still lower among Japanese in Hawaii and in Japan.

These observations indicate the need for new epidemiologic data that emphasize differences of race, culture, ways of life, stress and strain, and dietary habits. It is the purpose of this paper to determine the role of environmental and nutritional factors in the genesis of coronary heart disease from an analysis of case records of patients treated in hospitals and private practice as well as field surveys of the general population belonging to different socioeconomic groups. Such an approach may supply a few clues to investigators, who may then study these problems in the laboratory.

Material

These studies have been conducted at Agra, one of the 3 big medical centers of Uttar Pradesh—the biggest state of India. The patients are drawn to this center not only from the rural and urban population of Agra, but from most of the surrounding western districts of the State.

The material for these investigations has been obtained from 2 sources: (1) case records of coronary heart disease patients, and (2) surveys of the general population.

1. The clinical material for this study was drawn from both hospital and private cases of coronary heart disease seen by the author[11] from January 1946 to August 1957. There were 553 cases of coronary heart disease among 3,273 cardiac patients during this period. These included 401 or 35.2 per cent of 1,127 private and 152 or 7 per cent of 2,145 hospital cardiac cases, giving

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an over-all incidence of 16.9 per cent in the combined private and hospital series. Of these 553 patients, 391 or 71 per cent were cases of myocardial infarction, 133 or 24 per cent were angina pectoris, and 27 or 5 per cent were cases of coronary failure.

The peak age incidence in the present series was 51 to 55 years (table 1). About two thirds of all cases between 40 and 60 years, 12.3 per cent were below 40 years, and the youngest was 18 years old.

There were 492 men (89 per cent) and 61 women (11 per cent), the sex ratio being 8:1 The sex difference was less marked after the age of 45 years.

The constitution of the patients was classified as ectomorphic in 39 per cent, mesomorphic in 51.3 per cent, and endomorphic in 9.7 per cent. Hypertension was present in 50.2 per cent, diabetes in 13 per cent; and the serologic test for syphilis was positive in 3.8 per cent.

A family history of coronary heart disease was available in 7.4 per cent, of hypertension in 3.8 per cent, and of diabetes in 2 per cent of the cases. These figures do not reflect the true familial incidence of these diseases, since most of them had been treated by practitioners of indigenous medicine who did not diagnose these diseases, and some others were ignorant of the cause of death among their close relatives.

2. The data concerning the general population have been obtained from records of a planned field survey[12] of the incidence of coronary heart disease including clinical and electrocardiographic studies, detailed analysis of the dietary pattern, and estimation of the blood lipids among 1,056 persons of the general population over the age of 25 years belonging to different socioeconomic groups.

In deciding on the socioeconomic classification, consideration was given to vocation, education, status in society, and income. Doctors, lawyers, university professors, high government officials, and businessmen with a monthly income over Rs. 500 per month were classed as that "high-socioeconomic group"; clerks, school teachers, petty officers, shopkeepers with income levels between Rs. 100 and Rs. 500 per month were classed as the "middle-socioeconomic group"; and industrial, manual, and agricultural workers with less than Rs. 100 as their monthly wages were grouped together as belonging to the "low-socioeconomic group."

During the course of the present investigations 216 of the high-, 350 of the middle-, and 480 persons of the low-socioeconomic group were studied. In the last category were included 230 industrial, 121 manual, and 129 agricultural workers. Eleven cases of coronary heart disease were encountered, giving an over-all incidence of 1.04 per cent.

Table 1

Age Distribution of 553 Cases of Coronary Heart Disease

Age group (years)	Number	Percentage
Below 20	1	0.1
21—25	4	0.7
26—30	10	1.8
31—35	22	4.0
36—40	32	5.8
41—45	67	12.1
46—50	101	18.3
51—55	113	20.4
56—60	76	13.7
61—65	64	11.5
66—70	39	7.1
71—75	18	3.2
76—80	4	0.7
Over 80	2	0.3

Observations

Urban Versus Rural

Of the 553 cases of coronary heart disease seen by the author 488, or 88.2 per cent, were from Agra and neighboring cities and towns, while the remaining 67 patients, or 11.8 per cent, came from villages.

One explanation may be that patients from rural areas are less likely to travel to Agra for an episode of chest pain, unless complications arise, whereas the city dwellers being conscious about heart attacks consult physicians on minor suspicion. The figures would not then give convincing proof of the relative frequency of coronary heart disease in urban and rural populations. The answer will come from field surveys. Although our experience of field studies in villages is limited, our impression is that the incidence is low in rural populations.

Socioeconomic Status

The incidence of coronary heart disease was 35.2 per cent of all cardiac cases in private practice and 7 per cent of cardiac patients admitted to the hospital. The majority of the former belong to the high-socioeconomic group and the majority of the latter come from the low-socioeconomic group. On further analysis

Table 2

Incidence of Coronary Heart Disease in Different Socioeconomic Groups of the General Population

Socioeconomic group	Number studied	Coronary heart disease	Percentage
High	216	9	4.0
Middle	350	2	0.6
Low	480	0	0
Industrial —230			
Manual —121			
Agricultural—129			

it was observed that of the 553 cases of coronary heart disease 63.3 per cent belonged to the high-, 25 per cent belonged to the middle-, and 11.7 per cent to the low-socioeconomic group.

In the field studies among 1,056 unselected individuals, the incidence of coronary heart disease was 4.1 per cent in the high group, and 0.57 per cent in the middle group. No case was encountered among 480 industrial, manual, and agricultural workers (table 2).

These observations emphasize the marked preponderance of coronary heart disease in the privileged class of society in contrast to the socioeconomically backward class, which enjoys a relative immunity from this disease.

Diet

Of the 553 cases 62.2 per cent were vegetarians and 37.8 per cent were nonvegetarians. But this question is unimportant in India because, with few exceptions, the so-called nonvegetarians in India take meat only 2 or 3 times a week.

We have therefore studied the nutritional status of 102 consecutive cases of coronary heart disease with regard to daily intake of fat, proteins, carbohydrate, cholesterol, total calories, and percentage of calories from fat and compared them with similar studies carried out in field surveys among normal persons belonging to various socioeconomic groups.[13] The focal point of interest in the diet has been the relationship of dietary fat to coronary heart disease.

The maximum incidence of coronary heart disease in the field survey was found in persons of the high-socioeconomic group who consume the highest amount of total dietary fat and derive the highest percentage of calories from dietary fat. Members of the high-socioeconomic group consumed 86.5 per cent of saturated fat versus 77.7 per cent by those in the middle and 52.1 per cent in the low groups.

The fat intake, percentage of fat calories and saturated fat in the dietary pattern of coronary cases are similar to the high-socioeconomic group of society, which bears the maximum burden of coronary heart disease.

Serum Lipids

Studies of serum lipid in the field survey gave the highest values of serum cholesterol in the high-socioeconomic group and lowest in the low-socioeconomic group; this was also true for the values of serum lipid phosphorus. The cholesterol/phospholipid ratio in the 3 groups did not differ significantly from one to another. A gradual rise in serum cholesterol was also observed with age from 20 to 50 years, after which a slight fall occurred.[14]

Eighty normal controls were drawn from this study and matched according to age, sex, and socioeconomic status with 80 cases of coronary heart disease, and their serum lipids were compared.[15]

The mean values of total serum cholesterol, serum lipid phosphorus, and cholesterol/phospholipid ratio were significantly higher in the coronary group than in the control group. In cases of coronary heart disease, the rise in total serum cholesterol was greater than the rise in serum lipid phosphorus. There was a considerable overlap of values between the coronary group and the normal group but very high values were infrequent in normal individuals.

It is considered that determination of serum cholesterol is a reliable and simple procedure for identifying persons with gross disturbance of lipid metabolism, which is blamed as one of the factors in coronary heart disease.

Physical Activity

Business executives formed the largest percentage of 553 patients with coronary heart disease, with doctors, landlords, and high go-

rnment officials following in order. Shopeepers, clerks, and petty officers accounted or a smaller group of such patients. The inimum number of cases were found among aborers and farmers. The results of the field urvey confirmed the observations made in he hospital wards and private practice.

In an evaluation of the amount of physical etivity involved in each case, it was roughly ssumed that the higher strata of society, which bear the maximum burden of coronary eart disease, did little manual work in narked contrast to the poorer sections of soiety, whose work and mode of life demand greater amount of physical work.

Stress and Strain

It is difficult to assess the part played by stress and strain on the incidence of coronary heart disease in an epidemiologic study of this nature. The emotional tempo of life has gone up considerably during the last 2 decades, and this is more evident in the big cities. The poor Indian villager is quite removed from this tension and relaxes more completely when away from his work; that may partly explain his relative protection from coronary heart disease.

Smoking and Alcohol

Our studies fail to establish any relation of smoking or alcohol to coronary heart disease. Of 553 cases, only 149 or 26.9 per cent smoked and 62 or 11.2 per cent took alcohol, most of them moderately.

Discussion

Published reports on coronary heart disease from India are few.[6, 8, 9, 16] Their observations on the relationship of age, sex, and body build to its frequency are similar. Its high incidence in upper class and urban population has been reported. The conclusion drawn by Malhotra[9] that vegetarians were no less predisposed to coronary heart disease than nonvegetarians has not much significance. Environmental factors with special reference to socioeconomic status and their relationship to the dietary pattern and blood lipids in cases of coronary heart disease as against the normal population are being presented from India for the first time.

Table 3

Dietary Factors in Different Socioeconomic Groups of General Population and Cases of Coronary Heart Disease

	Daily fat intake (Gm.)	Percentage calories from fats	Percentage saturated fat
Coronary heart disease	69.1±29.1	29.1± 4.2	82.8± 9.6
High-socio-economic group	77.1±25.8	32.8± 9.4	86.5±17.9
Middle-socio-economic group	60.8±22.8	20.9± 8.6	77.7±15.3
Low-socio-economic group			
Industrial	51.8±19.3	15.1±13.8	52.1±16.0
Manual	40.4±14.2	16.9± 4.9	49.0±14.3
Agricultural	56.6± 6.9	22.4± 5.7	59.6±24.3

A recent development in India has been the field studies of general population such as the one reported here. The only other parallel study is being conducted by Padmavati et al.[17] at Delhi. She reported an incidence of 4 per cent in the high-socioeconomic group in contrast to 0.5 per cent in the rural population of Nazafgarh, and 0.17 per cent in the industrial workers. The corresponding incidence in the high-socioeconomic group in the present series was 4.1 per cent in the survey of the general population.

It seems clear from the studies on patients and the general population that coronary heart disease has a predilection for the privileged class of society in great contradistinction to individuals of low-socioeconomic group, who enjoy relative immunity from this disease. How these socioeconomic factors operate has been a baffling question in the highly complicated problem of coronary heart disease.

The answer to the relationship of diet to coronary heart disease has been sought in epidemiologic surveys, clinical studies, and metabolic ward experiments. The highest incidence of coronary heart disease has been observed in members of the high-socioeconomic group, who consume the highest amount of

Table 4
Mean Lipid Values in Cases of Coronary Heart Disease and Normal Controls

Serum lipids	Coronary heart disease	Normal
Total serum cholesterol (mg. %)	218±52.4 (114-350)	182.6±38.2 (96-275)
Serum lipid phosphorus (mg. %)	9.46±1.92 (5.3-14)	8.44±2.49 (5-11)
C/P ratio	24.1±3.21 (19.8-29.4)	21.7±2.18 (17-27.2)

dietary fat, the highest percentage of fat calories and saturated fat. But on subgrouping of each socioeconomic group on the basis of dietary fat intake, it was observed that the serum lipid level did not show a corresponding rise with the rise in dietary fat consumption.[13]

Although the total quantity of fat, percentage of calories contributed by fat, and percentage of saturated fat may have an important relationship with coronary heart disease, other factors like total caloric intake, relative rate of caloric expenditure, physical activity, emotional status, and hormonal influences cannot be easily isolated from the problem of excessive fat intake. Consequently the epidemiologic studies do not give a convincing answer to the relationship of dietary fat and coronary heart disease.

In India the dietary habits of people have not changed significantly during the last decade or two, but with the advance of modernization, industrialization, and urbanization, physical labor has been curtailed considerably, and people are exposed to greater stress and strain. These may be important factors in the increasing incidence of coronary heart disease.

Summary

Various factors relating to coronary heart disease were investigated in 2 groups of patients: 553 patients with clinical coronary heart disease and 1,056 persons selected in a field survey of the general population.

The incidence of coronary disease was high in the urban population, in the upper socioeconomic classes, and in persons with highest amounts of total dietary fat and highest percentage of calories from dietary fat. Total serum cholesterol, serum phospholipid, and cholesterol/phospholipid ratio were higher in the coronary group.

Finally, it seemed that physical activity was less and emotional stress and strain greater in the patients with coronary disease.

No relation between coronary disease and smoking or alcohol was established.

Summario in Interlingua

Varie factores relationate a morbo cardiac coronari esseva investigate in 2 gruppos de patientes. Le prime consisteva de 553 patientes con clinicamente manifeste morbo cardiac coronari, le secunde consisteva de 1.056 subjectos seligite in un investigation profilatori del population general.

Le incidentia de morbo coronari esseva alte in le population urban, in le classes socio-economicamente superior, e in gruppos de subjectos con le plus alte totales de grassia dietari e le plus alte procentages de calorias ab grassia deitari. Le valores pro cholesterol total del sero, pro phospholipido seral, e pro le proportion de cholesterol a phospholipido esseva plus alte in le gruppo coronari.

Finalmente, il pareva que le activate physic esseva inferior e que le stress emotional esseva superior in le patientes con morbo coronari.

Nulle relation esseva constatate inter morbo coronari e le uso de taboco e de alcohol.

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An Awareness of the History of Medicine

A physician is inevitably wrapped in the fabric of the history of medicine, for in his daily undertakings he comes face to face with the experience of his predecessors who effectively pursued the art and the science of both practice and research. The observational and constructive methods that he uses in creating a case through careful history taking may bring to his mind, if he is well oriented, the contributions of Plato, Aristotle, Hippocrates, Galen, Maimonides, Vesalius, Harvey, Leeuwenhoek, Boyle, Pasteur, Virchow, Sydenham and many others.

* * *

It is a way of building a bridge between the practice of medicine and the humanity of science. There are distinguished physicians today who, though preoccupied with immediately practical or scientific problems, reap a sense of deep personal satisfaction in following the long course of medical history back through the Renaissance and the Middle Ages to Greco-Roman periods of thought and action. . . . The exercise of the intellect in this manner will focus his attention on the deductive and inductive methods, the dogmas, the tenuous speculations, the working hypotheses and the experiments themselves that led to the growth or the retardation of medical history.

Paul, the apostle, in his first epistle to the Thessalonians uttered the well known thought, "Prove all things; hold fast that which is good." This counsel is particularly applicable to those who tend to worship the present and to scorn the past. The present may indeed represent the high, immediate mountain view of medicine, but for all that the valleys of the past and the smaller mountain ranges that confine them are willy-nilly part of the present. A mere awareness of Greek, Hebraic, Arabic and Christian traditions as they relate to the present will give a physician a sense of pride in his calling and a feeling of being nearly complete in his total capabilities as he concerns himself with ethics, scientific education or patient-physician relations.—Editorial. *New England Journal of Medicine* 257: 290, 1957. (Reprinted with permission of the Publishers.)

Pathogenesis and Management of Chronic Ulcerations of the Extremities

By SHELDON G. SHEPS, M.D., F.R.C.P.(C)

A SIGNIFICANT number of chronic ulcerations of the skin of the extremities are due to specific diseases of the blood, the small blood vessels, and mesenchymal tissues. It is important to differentiate these conditions from the more common ulcerations secondary to venous stasis or major arterial insufficiency because the prognosis and treatment varies with the underlying disorder. Because these diseases are often overlooked, emphasis will be placed on their diagnosis and management.

Classification

Chronic skin ulcerations are best categorized on a pathogenetic basis. Most ulcerations are easily classified in this manner although not infrequently more than one pathologic substrate is found. In the following discussion, the mechanisms of ulcer formation will be highlighted.

Major Arterial Insufficiency

In conditions in which there is diminished inflow of blood because of major arterial obstruction there may be inadequate nutrition for resting metabolic needs. In the presence of severe reduction in blood flow, minimal trauma or infection can initiate local tissue necrosis and ulceration. Since the effectiveness of collateral blood supply will vary with the size of the artery and length of the obstructions, ischemic ulcers are usually seen in distal areas; i.e., toes, heels, fingertips. These ulcers are characterized by severe local pain, often relieved by dependency. The pain has been ascribed both to tissue anoxia and also to irritation of pain-sensitive nerves. Usually there is no systemic disturbance unless there is severe secondary infection. This complication is seen especially in patients with diabetes. The ulcers themselves may be of any size (fig 1). The edges are often irregular and the center is frequently covered with a tough eschar. The granulations are pale and boggy. Marked inflammation is uncommon because of the poor blood supply. Complete obstruction of major arteries is always present proximal to the ulcerated area. Postural color changes indicating arterial insufficiency are present, and there is a delay in venous filling in the dependent limb. Diseases characterized by major arterial obstruction include arteriosclerosis obliterans, thromboembolism, and thromboangiitis obliterans.[1, 2] In addition, atherosclerosis may involve the small arteries as well as the large ones, since atheromata can be seen in arteries as small as 500 to 900 μ in diameter.[3]

Venous Stasis

Insufficient deep or superficial veins of any origin encourage the pooling and stagnation of blood when the involved area is in a dependent position. Arteriovenous fistulas may produce a similar picture. If the fistulas arise before the epiphyses close, the limb is larger and longer than its mate. Bruits may be heard over the involved limb, and it is warmer than its opposite.

Longstanding stasis produces induration and fibrosis of the skin and subcutaneous tissues, which is often associated with brown pigmentation. These deposits contain both hemosiderin and melanin. The thickened areas are less resilient than normal skin, and minor degrees of injury produce superficial ulceration. These ulcers are usually confined to the lower leg (fig. 2). They are ordinarily located on the lower third of the medial aspect of the leg, especially over the medial malleolus. They are of irregular shape and variable size. The ulcers are shallow and often are covered with a dirty exudate. There are no systemic signs

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of inflammation. Occasionally ulceration occurs following direct injury to a varix. Ordinarily pain is not severe and it is relieved by elevation and external compression.

Diseases of Small Vessels (Arterioles, Capillaries, and Venules)

This is a large and heterogeneous group of conditions in which skin ulceration is but one manifestation of the disease.[3] The largest number belongs to a pathologically similar group with differing clinical manifestations that includes livedo reticularis, acrocyanosis, chronic pernio, post-cold-injury syndrome and advanced Raynaud's disease. Spontaneous nodular lesions and ulcers may occur in the extremities in all of these conditions. The appearance of the early lesion and its subsequent course suggests infarction of the skin and the subsequent ulcer has an ischemic appearance. Biopsy in all these conditions reveals angiitis involving the entire blood vessel wall, necrosis of subcutaneous fat, and chronic inflammation in subcutaneous tissues with the occasional presence of giant cells. Hypertensive ischemic ulcers and ulcerations seen in patients with rheumatoid arthritis are similar in gross appearance to this group, but histologic examination of the tissues in hypertensive ischemic ulcers reveals chronic hypertensive changes in the small arterioles rather than angiitis; and in the ulcers seen in rheumatoid patients the small arteries demonstrate concentric thickening of the intima and secondary thrombosis with some inflammatory cells in the wall, but fibrinoid necrosis is absent.[4] Histologic lesions characteristic of the rheumatoid nodule have also been described.[5]

The typical early lesion consists of a pink or erythematous plaque in the skin about the size of a dime, which changes to a dusky blue or purpuric color in the course of a week or 10 days. A hemorrhagic blister may develop at this time. The necrotic area sloughs out leaving a superficial ulcer. The ulcer often has necrotic eschar remaining in the center surrounded by very poor granulation tissue. It is exquisitely sensitive and extremely painful. Longstanding ulcers are surrounded by brown

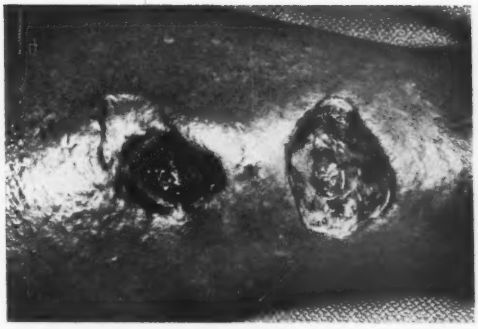

Figure 1

Typical ischemic ulcerations on the skin. The smaller one shows evidence of healing.

pigmentation due to the deposition of both hemosiderin and melanin.

Livedo reticularis is characterized by a network of reddish or cyanotic hue in the skin. It occurs in both sexes. It is occasionally associated with sensations of coldness, numbness, and aching. A cool environment enhances its appearance. Livedo is occasionally secondary to periarteritis nodosa, disseminated lupus erythematosus, and a variety of neurologic diseases. In the secondary form, the network is usually patchy. The idiopathic variety is diffuse and symmetrical, involving all extremities and, not uncommonly, the trunk as well. Ten per cent of these patients develop recurrent ulcerations, usually on the lower leg.

Acrocyanosis is a condition in which the hands and feet are persistently blue and cool, especially in a cold environment. The discoloration is diffuse in contrast to the network seen in livedo reticularis. In addition, this is seen almost exclusively in females. Occasionally it is associated with an increase in sweating and paresthesias. At times, exposure to cold may induce edema and tenderness. Nevertheless, blanching, trophic changes, and gangrene are never seen. Local trauma may produce an ulceration but this always heals. Acrocyanosis is occasionally seen secondary to pulmonary osteoarthropathy.

Chronic pernio or chilblains is usually seen in women in the fourth and fifth decades. It occurs where exposure to cold temperatures

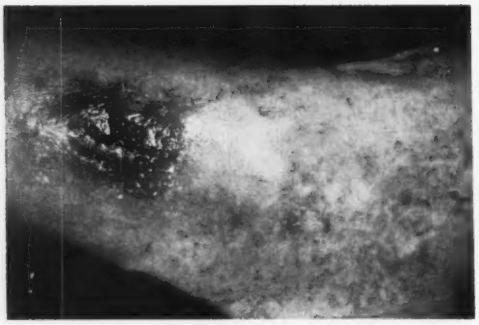

Figure 2

Typical superficial ulceration due to venous stasis in an area of pigmentation just above the medial malleolus.

occurs at some time during the year. These women often suffer from lipedema as well. The lesions appear in crops in a fairly characteristic cycle; i.e., the ulcers develop over a period of 2 or 3 weeks and then heal over a similar period of time, resolving into a pigmented and often depressed scar (fig. 3). They occur in the fall and winter at first, and sometimes even cool summer days can induce a crop of ulcerations. These ulcers are always painful and occur about the ankles. After a number of years the skin becomes indurated, the ulcers become indolent, and the seasonal element is lost. It is important to prevent the development of the chronic phase, which is very difficult to manage.

Chronic pernio must be differentiated from 3 other nodular lesions that occasionally produce ulcerations. *Erythema induratum* occurs in symmetrical crops, especially on the calves. It may be seasonal like chronic pernio. The early lesions are more nodular and indurated than in pernio. It is always associated with tuberculosis elsewhere in the body, and histologic examination of the area reveals tubercle formation and caseation. These ulcerations heal with a depigmented scar, which may be surrounded by a ring of hyperpigmentation. *Nodular vasculitis* (nontuberculous erythema induratum) occurs in similar areas. It also affects women in their thirties and forties. However, the lesions are more active and nodular than either erythema induratum or chronic pernio and very rarely produce ulceration. There is no relationship to environmental temperature. The lesions appear either singly or as crops of nodules, which may persist for several months. Histologic examination of lesions in this condition reveals a very active vasculitis. *Erythema nodosum* is an acute systemic process which often occurs in the spring and fall and lasts from 2 to 6 weeks. It is characterized by the bilateral appearance of pea-sized nodules, usually along the anterior tibial border. These very rarely ulcerate.

Hypertensive ischemic ulcerations have been recognized for only some 20 years. They usually occur in women in the sixth and seventh decades who have a history of longstanding moderate or severe hypertension. These patients may be normotensive following a myocardial infarction or cerebrovascular accident, for example, but funduscopic examination reveals hypertensive changes in the arterioles. The ulcerations always occur in the leg and usually posterolaterally on the calf, especially about the lateral malleolus (fig. 4). Often they are solitary, but occasionally may be multiple. Their size varies from 1 to 15 cm. in diameter. They occur spontaneously but may be secondary to injury. They tend to extend by purpuric infarction of the skin bordering the ulcer. Characteristically they are extremely painful and often require prolonged administration of narcotics for alleviation of pain. These ulcers often take many weeks or months to heal spontaneously. Recently, *rheumatoid arthritis* was reported associated with similar stubborn ulcerations.[4, 5] These conditions should be considered in all patients with ischemic ulceration.

Trench foot, immersion foot, and their more severe counterpart *frostbite,* may be followed by a chronic vasospastic disorder. In more severe cases, coldness, pain, stiffness, and other paresthesias of the involved extremities persist. These limbs are cold and cyanotic even in a warm environment. Raynaud's phenomenon may also occur. There is other evidence of vasomotor instability in the form of hyperhydrosis. Sensitivity to cold is prominent.

Occasionally this vasospastic ischemic phase is associated with localized areas of gangrene. These are always superficial and when the necrotic tissue sloughs away, there is healthy tissue underneath.

Raynaud's phenomenon may be defined as episodic constrictions of the small arteries or arterioles of the skin of the fingers and toes and occasionally ears, nose, and lips, resulting in intermittent changes in the color of the skin in these areas.[6] When the occlusion is complete, pallor results; when it is incomplete, the skin becomes cyanotic. The episodes of constriction are followed by reactive hyperemia that produces a red color (rubor). Each phase lasts a few seconds to a few minutes. Individuals differ as to whether they experience pallor or cyanosis alone or consecutively. In addition, the reactive hyperemia is also inconstant; but when it does occur, it always follows an episode of vasospasm. When Raynaud's phenomenon is "primary" it is called *Raynaud's disease.* This usually occurs in women and involves the fingers of *both* hands with few exceptions. The majority of patients are under 40 years of age at the time of onset. Exposure to cold is the usual stimulus for precipitating Raynaud's phenomenon but emotional stress occasionally may serve as a trigger. Some 30 per cent eventually develop trophic changes, sclerodactylia, and calcinosis of the fingertips. These may appear separately or together. The trophic changes occur in the tips of the digits, about the nails and in the web spaces. These changes include chronic fissures, superficial infarcts, ulcers, and chronic paronychia. The gangrenous areas are always minimal and rarely lead to amputation. If they do, the operation is always minor, involving no more than 1 or 2 phalanges.

Raynaud's phenomenon may be *secondary* to a variety of disorders.[6] The following conditions must be excluded before a diagnosis of *primary* Raynaud's disease can be made: arterial occlusive disease, neurogenic disease, hematologic disorders, "collagen" diseases, repetitive trauma to the hands, and intoxication with ergot or heavy metals. All these conditions may be complicated by trophic changes and ulceration, etc., just as in Raynaud's disease. However, treatment of the secondary variety is less effective than in the group with primary Raynaud's disease.

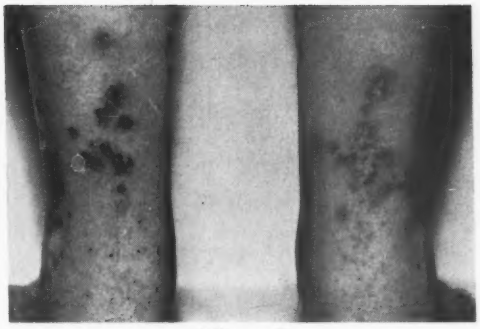

Figure 3

Classical chronic pernio demonstrating scars from healed lesions and a crop of fresh ulcerations. (Reproduced by courtesy of the Vascular Section, Mayo Clinic, Rochester, Minn.)

Ulceration Seen in Various Blood Dyscrasias

In the *hemoglobinopathies,* chronic ulcers may be found as part of the clinical picture.[7] These conditions include sickle-cell disease, Cooley's anemia, and hereditary spherocytic anemia. The ulcerations usually appear in the third decade of life and are associated with a chronic anemia. They may be more common in women. The ulcers are recurrent and indolent, but relatively painless. They are often localized about the ankle and foot. They may occur in an area of brown pigmentation. The mechanism of ulcer formation has been ascribed to stasis combined with hypoxia.

In the myelo-proliferative disorders, skin infarction may occur because of a hypercoagulable state with or without an increase in the number of platelets. It also may be associated with disease of the small arteries and arterioles.[8]

Diseases of the blood associated with secondary Raynaud's phenomenon may also be included in this group of disorders; i.e., paroxysmal hemoglobinurias; a high titer of cold hemagglutinins or cryoglobulins.

Neurotropic Ulcers

When pain sensation is reduced or absent, minor injury is often overlooked and may lead

Figure 4

Typical hypertensive ischemic ulcerations proximal and posterior to the lateral malleolus.

to infection and ulceration. These injuries usually occur at the sites of pressure and often under calluses. These ulcers appear punched out and, of course, are relatively painless. Such ulcers are seen in tabes, whether of syphilitic or diabetic origin, spina bifida, syringomyelia, and other diseases of the central and peripheral nervous system, and leprosy. Diabetes mellitus is in a special category because a neuropathy or myelopathy may be associated with rather severe arterial and arteriolar occlusive disease. Many already have lost neurogenic control of blood vessels (autosympathectomy).

Specific Skin Ulcers

This group includes the specific infections of the skin that are associated with chronic ulcers; e.g., blastomycosis, a variety of tropical sores, syphilitic granuloma,[9] etc. Malignancies of the skin may also be included in this group; e.g., basal-cell carcinoma, epithelioma, mycosis fungoides.

Factitial Ulcers

The use of certain ointments and solutions, whether applied by the patient or his physician, often impairs healing of wounds. Many contain irritants such as iodine, merthiolate, and phenol, which can injure delicate granulation tissue and ischemic skin. In addition, scratching and continued mechanical trauma may play a part in maintaining chronicity. Decubitus ulcers may also be included in this group.

Principles in the Treatment of Chronic Ulcers

Cleansing and Debridement of the Ulcerated Area

This is a most important phase of treatment. Bed rest with intermittent soaking of the ulcerated area with warm sterile solutions; (e.g., saturated solution of boric acid (4 per cent) 0.5 per cent aluminum subacetate, or saline), and complete change of the dressing 4 or 5 times a day to remove the loosened debris, will nearly always succeed in cleaning up an infected necrotic area. Difficult eschars may be attacked medically by enzymatic debridement, i.e., dressings of a solution or a water-miscible jelly containing streptokinase and streptodornase (Varidase).[2] In the absence of major arterial disease, local surgical debridement may be carried out. Other local salves and solutions usually produce maceration, which increases the size of the ulcer and delays healing. Furthermore, this increase in necrotic tissue decreases absorption of the "therapeutic" agent and therefore these ointments are usually ineffective.[2, 10, 11]

Promotion of Healing

1. Infection is combated by the *systemic* use of appropriate antibiotics after culture and sensitivity studies have been carried out on material taken from the ulcer. Antibiotics administered systemically reach the infected area of the most ischemic ulcers.[12] Thus the local reactions of topically applied antibiotics are avoided.

2. Local blood flow should be enhanced. This is facilitated by the use of vasodilating agents such as dibenzylene, ganglion-blocking drugs, alcohol, warm environment, etc. Regional sympathetic denervation (by surgery or injection of absolute alcohol) increases the blood supply to the *skin*, and is of value in certain situations; e.g., livedo reticularis, cold injury, chronic pernio. In this regard it is important to remember that many diabetic patients develop a myelo-neuropathy that often involves the sympathetic nervous system. The peripheral vascular beds may already be denervated. Therefore, before undertaking regional sympathetic denervation in diabetic patients, appropriate tests must be carried out

to demonstrate intact sympathetic fibers; e.g., skin temperature studies and sweat tests. Because of its vasoconstrictive effects, tobacco in any form is contraindicated.[3] Restoration of major arterial continuity is carried out where feasible, both technically and in relation to the general condition of the patient.[13]

When stasis and edema are prominent, elevation of the part involved enhances healing. On the other hand when there is major vascular occlusion, blood flow may be enhanced by a dependent position. Continuous maintenance of a dependent position tends to induce edema, so that in the instance of major vascular occlusions, the use of an oscillating bed for a period of 8 hours a day is very helpful.[2]

3. When the ulcers are clean and the granulation tissue is healthy, the application of dry, powdered blood cells may hasten healing. This forms a crust which is removed every 2 or 3 days by soaking with boric acid solutions as outlined earlier. For large ulcers, excision and skin grafting or skin grafting without excision reduces the period of disability.[10]

Treatment of the Underlying Condition

In the discussion of the pathogenesis of the various ulcers, the primary disease state has been defined. Management of this should proceed coincident with local therapy of the ulcer.[14-16] Prevention of further ulceration lies in the protection of involved areas from chemical, thermal, and mechanical injuries. In a few conditions, regional sympathetic denervation is of value in ulcer prophylaxis; e.g., chronic pernio, secondary acrocyanosis.

Treatment of Specific Ulcerations

Major Arterial Insufficiency

The management of these ulcerations includes protection from further chemical, thermal, and mechanical injury; local cleansing and debridement, systemic antibiotics to eradicate local infection; and enhancement of local blood supply by regional sympathetic denervation, vasodilator agents, and maintenance of a warm environment.[2] The use of a peripheral nerve crush or section often relieves much of the pain and allows more complete debridement—the site of incision must have a blood supply adequate for healing. The application of arterial bypass grafting where feasible has increased greatly the salvage of ischemic extremities.

Venous Stasis

With bed rest and elevation to reduce the edema, and the application of warm, moist dressings of 4 per cent boric acid or 0.5 per cent aluminum subacetate to clean the ulcers, healing takes place quite rapidly. Protection of the healed area with foam-rubber pads and prevention of edema by the addition of elastic supports to the leg are quite adequate to prevent further ulceration in many patients. When there is obvious insufficiency of veins draining into areas of ulceration, appropriate stripping of such veins and others similarly involved, with or without excision and grafting of the ulcerated areas, may be carried out with excellent results. The smaller ulcers are managed from the office, but hospitalization substantially reduces the period of morbidity in the remainder.

Diseases of Small Blood Vessels

The ulcers in this group usually respond to bed rest and local cleansing solutions. Vasodilator drugs, fever therapy, and ganglion-blocking agents may be administered in an attempt to hasten healing. They do not respond well to the application of any ointments or salves. Regional sympathetic denervation is rarely necessary. Amputation is very rarely required. In chronic pernio and post-cold-injury syndrome regional sympathetic denervation increases the tolerance to cold.

The treatment in the complicated phase of Raynaud's disease is much the same as that in the other diseases described in this section. An ointment containing 2 per cent glycerol trinitrate has been used for some time in the management of vasospastic phenomena in Raynaud's disease with questionable success. Sympathectomy is usually performed in the complicated phase. When adequate follow-up is carried out (10 years or more), it is beneficial in only half the cases operated upon.[6] Restriction of tobacco does not appear to influence the morbidity.[6]

Treatment of hypertensive ischemic ulcerations is quite protracted. When the ulcerations become clean and the granulation tissue looks healthy, excision and skin grafting is helpful in closing over the defects and lessening the morbidity. Occasionally regional sympathetic denervation will hasten healing. The hypertension should be treated at the same time.

Ulcers Associated with Blood Dyscrasias

The management of the ulcerations seen in the hemoglobinopathies is similar to that of venous stasis. Skin infarcts that ulcerate are treated in a similar fashion to hypertensive ischemic ulcerations. The underlying blood disorder should be attended to at the same time, if possible.

Neurotropic Ulcers

When there is no associated vascular disease, these ulcers usually heal following relief of pressure and debridement. Diabetic patients with associated arterial disease do not respond well to local treatment and often require some degree of amputation.

Summary

It can be seen that the variety of chronic ulcerations of the skin of the extremities is legion. Attention to the underlying disease process is of paramount importance because of the difference in definitive treatment and prognosis. Appropriate simple medical measures as outlined above often suffice in the local treatment of these ulcerations. Regional sympathetic denervation has a definite but relatively minor role in the management of these disorders. The widespread, indiscriminate use of a host of salves, ointments, and other potions is to be condemned.

Summario in Interlingua

Es apparente que le varietates del ulcerationes chronic del pelle in le extremitates es numerosissime. Il es del plus grande importantia que attention es prestate al subjacente processo pathologic a causa del differentias in le tractamento definitive e etiam in le prognose. Appropriate simple mesuras medical del character delineate in iste reporto suffice frequentemente pro le tractamento local de iste ulcerationes. Disnervation sympathic regional ha un definite sed relativemente minor rolo in le tractamento de iste disordines. Le extense non-discriminante uso de un vaste serie de emplastros, unguentos, e altere preparatos debe esser condemnate.

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Cerebral Hemodynamic and Metabolic Studies in Patients with Congestive Heart Failure

I. Observations in Lucid Subjects

By WILLIS SENSENBACH, M.D., LEONARD MADISON, M.D., AND SEYMOUR EISENBERG, M.D.

MEASUREMENTS of cerebral blood flow and metabolism in subjects with congestive heart failure have yielded conflicting results.[1-3] Scheinberg[1] compared the values in 14 persons with congestive heart failure of unstated severity with those in normal young men and concluded that cerebral blood flow was significantly decreased in subjects with cardiac decompensation. Novak and co-workers,[2] on the other hand, compared their findings in 15 decompensated cardiac patients with the values in a "similar arteriosclerotic group" and concluded that the cerebral circulation was not affected by the congestive state. In 20 hypertensive individuals with congestive heart failure Moyer and associates[3] found no significant diminution in cerebral perfusion, nor was there any significant change following treatment; cerebral blood flow, however, was apparently diminished in 6 subjects with valvular heart disease and congestive failure. In addition, Moyer's group[4] reported that the administration of aminophylline to persons with congestive heart failure resulted in a decline in cerebral blood flow.

In an attempt to resolve the existing confusion, the present study was undertaken. The design of the study permits comparison of cerebral blood flow in 2 types of patients with cardiac decompensation: those with mild forms and those with severe congestive failure. In addition, many patients were studied prior to treatment and again after compensation had been achieved. This experimental design was prompted by preliminary observations suggesting that alterations in cerebral hemodynamics and metabolism were largely contingent upon the severity of the congestive state.

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Materials and Methods

Thirty-seven men with congestive heart failure were studied. In every instance the diagnosis of congestive heart failure was established by the classical clinical configuration of breathlessness, edema, cardiomegaly, hepatomegaly, and venous distention. Persons with significant pulmonary disease, primary renal disease, anemia, or obvious cerebral arteriosclerosis were excluded from the study. The patients were studied in the recumbent posture, although the presence of orthopnea occasionally necessitated slight elevation of the head. In 14 subjects measurements were again made after compensation had been achieved. Therapy included digitalis, sodium restriction, and diuretic measures when necessary.

The patients have been classified according to the severity of the congestive state. For the majority of the subjects with mild to moderate failure this represented the first episode of cardiac decompensation. Edema was present in all patients, but the amount in these patients was modest in contrast to the massive anasarca of most severe cardiac patients. Only minimal cardiac enlargement was present in most of these patients and heart size tended to return to or toward normal following therapy. The response to digitalis and sodium restriction alone was prompt and gratifying in most instances; several of the patients were given diuretic agents to hasten the delivery of their edema but none required these agents to remain edema-free after compensation had been achieved. Individuals with severe congestive failure, on the other hand, had had repeated episodes of decompensation for a protracted period. The magnitude of cardiac enlargement was considerably greater than in the milder cardiac subjects, and the reduction in cardiac size following treatment was minimal in most instances. Rigid sodium restriction and frequently administered diuretic

agents were necessary to free these patients from edema; moreover, once optimum compensation had been achieved, all these patients required periodic diuretic therapy to remain edema-free. Dyspnea on exertion persisted in the majority of these subjects despite treatment, whereas patients with only mild decompensation were essentially symptom-free with moderate activity once compensation had been restored. All of the patients were lucid.

Cerebral blood flow was determined by the nitrous oxide method of Kety and Schmidt,[5] as modified by Scheinberg and Stead.[6] The analytic methods employed have been described in a previous report.[7] Cerebral oxygen consumption was calculated from the arterio-cerebral venous oxygen difference and cerebral blood flow. Cerebral vascular resistance was calculated from the cerebral blood flow and the mean arterial blood pressure. The latter was measured directly from a peripheral artery by a damped mercury manometer. Arterial blood samples for pH determination were drawn anaerobically and the pH was immediately determined at 37 C. by means of a Cambridge Research Model pH Meter. Carbon dioxide tension was calculated from the nomogram of Singer and Hastings.[8]

Results

The results in patients with congestive heart failure of mild to moderate severity are presented in tables 1 and 2 and those in persons with severe failure in tables 3 and 4. The 14 subjects who were studied prior to treatment and after optimal compensation was achieved are appropriately indicated. In table 1 are presented normal values for cerebral hemodynamic and metabolic functions in elderly subjects; these are mean values from the laboratories of the indicated investigators. The mean age in most of these studies was considerably greater than that of the subjects comprising the present report; therefore, the results in the present study have not been compared statistically with these "normal values."

Cerebral blood flow was significantly decreased (39 ml. per minute per 100 Gm.) in persons with severe congestive heart failure but was "normal" (51 ml. per minute per 100 Gm.) in the group with mild to moderate decompensation. The mean arterial blood pressure was 21 mm. greater ($p<.05$) in the group with mild to moderate failure. The cerebral oxygen consumption was 3.8 ml. per minute per 100 Gm. in the milder cardiac subjects as compared with 3.24 ml. in the group with severe decompensation. Although this difference was statistically significant, ($p<.02$) both values were "normal" or slightly increased for people in this age group.[9-12]

In 10 persons with mild to moderate cardiac decompensation, relief of the congestive state was associated with a significant ($p<.01$) decline in cerebral blood flow. Mean arterial blood pressure also decreased significantly ($p<.02$); cerebral vascular resistance, on the other hand, remained unchanged. Cerebral oxygen consumption decreased slightly, but this change was not significant. In addition, arterial oxygen content increased significantly ($p<.01$) following treatment as did venous carbon dioxide content. Only 4 patients with severe congestive heart failure were studied following treatment; significant hemodynamic alterations were not apparent from this meager sample.

Discussion

The results of this study clearly demonstrate that congestive heart failure is associated with significant cerebral hemodynamic alterations. The earliest and most consistent abnormality in these patients was a significant increase in cerebral vascular resistance. Cerebral blood flow, on the other hand, was apparently maintained at normal levels in the face of this increased resistance until severe congestive failure supervened, whereupon cerebral blood flow decreased significantly. This reduction in blood flow with worsening decompensation was not accompanied by further increments in vascular resistance but was associated with a significantly lower mean arterial blood pressure.

Cerebral vascular tone is largely governed by changes in the gaseous composition or acid-base equilibrium of arterial blood. As cerebral vasoconstriction occurred in these individuals in the absence of such changes, it seems likely that the increase in resistance in these vessels was the result of hemodynamic alterations secondary to the supervention of myocardial

Table 1

Cerebral Circulatory and Metabolic Functions in Patients with Mild to Moderate Congestive Heart Failure

Name	Age	Diagnosis	CBF ml./min./100 Gm.		MABP mm. Hg		CVR ml./min./100 Gm. 100 Gm.		$CMRO_2$ ml./min./100 Gm.		CMR Gluc. mg./min./100 Gm.	
Kety[9, 10]	80	Normal	47.7		94		2.1		2.7			
Kety[9]	68	HVD	48.7		150		3.2		2.8			
Scheinberg[11]	63	Normal	50.6				2.03		3.3			
Shenken[12]	68	Normal	46				2.1		2.7			
			Decomp.	Comp.	Decomp.	Comp.	Decomp.	Comp.	Decomp.	Comp.	Decomp.	Comp.
O.B.	59	MI	49		94		1.92		4.40		4.41	
J.C.	64	MI	56		119		2.13		3.66		4.48	
E.W.	57	AI	48	45	106	82	2.21	1.82	5.22	3.97		3.15
J.C.	77	ASHD	56	45	106	116	1.89	2.58	3.91	2.95	5.04	4.95
T.H.	63	ASHD	55	50	81	69	1.47	1.38	3.80	3.70		
F.G.	60	HCVD	46	34	120	95	2.60	2.80	4.10	3.20		
G.P.D.	59	HCVD	46	35	157	122	3.41	3.49	2.86	3.54	2.30	4.90
H.T.	57	ASHD		37		88		2.38		1.92		3.70
R.H.	59	HCVD, ASHD	37	41	150	136	4.05	3.32	4.12	3.59	5.92	4.10
W.W.I.	64	ASHD	52	37	92	94	1.77	2.54	4.18	3.67	5.20	4.07
J.C.B.	62	HCVD	57		139		2.44		3.72			
A.T.	63	HCVD	73	58	169	140	2.32	2.41	4.90	4.34	3.65	2.90
A.E.N.	61	HCVD, ASHD	75		148		1.97		4.59		6.75	
W.B.	63	HCVD	44		123		2.80		3.20		5.72	
A.R.	48	HCVD	50		133		2.66		3.32		6.00	
L.C.	50	HCVD	49	30	136	104	2.78	3.47	4.03	2.64	3.92	3.90
J.C.	57	HCVD	44		91		2.07		3.78		4.40	
H.G.	42	ASHD	38		89		2.34		2.83		4.56	
A.R.McC.	57	ASHD		41		75		1.83		3.61		3.69
C.M.D.	60	AI	44		136		3.09		3.30		5.72	
M.C.	59	AS	70		64		0.91		3.14		2.80	
L.C.	53	HCVD	36		153		4.25		3.46		4.32	
D.P.	35	HCVD	39		173		4.40		3.98		3.51	
H.O.	51	ASHD	60	64	81	80	1.35	1.25	3.01	4.14	2.40	3.20
Mean	58		51	43	121	100	2.49	2.44	3.80	3.43	4.51	3.86
S.E.			2.40	2.90	6.61	7.14	0.194	0.220	0.135	0.195	.2988	
			Comparison of pretreatment and posttreatment values (10 patients)									
Mean difference				−8.3		−16		−.121		−.439		−.06
S.E.				2.526		4.915		.193		.256		.543
p				$<.01$		$<.01$		$>.5$		$>.1$		$>.5$

CBF, cerebral blood flow; MABP, mean arterial blood pressure; CVR, cerebral vascular resistance; $CMRO_2$, cerebral consumption of oxygen; CMR Gluc., cerebral consumption of glucose.

Decomp., decompensated; Comp., compensated.

AI, aortic insufficiency; AS, aortic stenosis; ASHD, arteriosclerotic heart disease; HCVD, hypertensive cardiovascular disease; HVD, hypertensive vascular disease; MI, mitral insufficiency; MS, mitral stenosis.

failure. It is conceivable, of course, that venous hypertension and cerebral congestion could have contributed to the total resistance to blood flow in the brain. The intense cerebral edema associated with superior vena caval obstruction, however, is not accompanied by cerebral hemodynamic changes,[13] and the increase in venous pressure under such circumstances is much greater than that encountered in subjects with congestive heart failure.

Table 2

Blood Constituents in Patients with Mild to Moderate Congestive Heart Failure

Name	Arterial O_2 content vol. %		Arterio-cerebral venous O_2 diff., vol. %		Arterial CO_2 content, vol. %		Arterial pH		Arterial pCO_2	
	Decomp.	Comp.	Decomp.	Comp.	Decomp.	Comp.	Decomp.	Comp.	Decomp.	Comp.
O.B.	18.01		8.97		43.13		7.44		36	
J.C.	16.90		6.53		46.20		7.41		38	
B.W.	16.65	17.97	10.88	8.82	32.93	42.81	7.38	9.39	30	38
J.C.	15.35	17.44	6.98	6.56	48.59	55.26	7.35		45	
T.H.	14.63	16.40	6.94	7.40	47.70	55.93			33	37
F.G.	20.21	20.51	8.90	9.36	43.80	45.44	7.61			
G.P.D.	19.46	19.40	6.22	10.12	43.48	37.55		7.47		28
H.T.		16.91		5.20		52.16		7.34		53
R.H.	21.00	21.19	11.13	8.75	45.95	46.98	7.51	7.39	32	41
W.W.I.	17.77	20.17	8.03	9.91	46.98	46.09	7.38	7.42	43	40
J.C.B.	19.39		6.52		48.46				42	
A.T.	16.67	17.39	6.71	7.48	47.28	50.04		7.42		44
A.E.N.	15.83		6.12		48.35					
W.B.	15.28		7.27		40.16		7.38		44	
A.R.	18.67		6.63		46.87		7.36		44	
L.C.	19.36	22.50	8.22	8.79	39.26	42.23	7.46	7.36	31	34
J.C.	16.65		8.58		35.85		7.42		29	
H.G.	14.93		7.46		45.35		7.47		35	
A.R.McC.		17.17		8.80		40.08		7.40		35
C.M.D.	16.67		7.49		42.51		7.41		36	
M.C.	15.79		4.48		59.37		7.39		53	
L.C.	15.71		9.62		42.35		7.48		30	
D.P.	19.95		10.20		41.14		7.37		39	
H.O.	15.21	18.52	5.02	6.47	51.05	53.03	7.39		45	
Mean	17.27	18.8	7.68	8.13	44.85	47.3	7.42	7.40	38.0	
S.E.	0.413	0.558	0.373	0.436	1.16	1.74			1.50	
	Comparison of pretreatment and posttreatment values (10 patients)									
Mean difference		1.52		.463		2.83		−.04		4.33
S.E.		.386		.578		1.451		.0397		1.744
p		$<.01$		$>.4$		$>.05$		$>.3$		$>.05$

Therefore, regardless of the degree of cerebral congestion, it seems likely that the cerebral blood vessels merely participate in the generalized increase in vascular tone that occurs in persons with cardiac decompensation, the cause of which remains obscure.

These data indicate that cerebral blood flow decreases with progressive deterioration of cardiac efficiency. It is conceivable that differences in the etiology of the heart disease in the 2 groups was partially responsible for the difference in cerebral perfusion, as the mild to moderate group included a greater percentage of hypertensive subjects. Moreover, the mean difference in cerebral blood flow between the 2 groups was obviously affected by isolated extreme values. Regardless of these possible influences and the overlap in values between the 2 groups, the majority of the individual observations tended to justify the conclusion that the decline in cerebral blood flow parallels the increase in severity of the congestive failure. It is not completely clear why blood flow decreases with deterioration. It has been observed that renal[14] and hepatic[15] perfusion tend to parallel cardiac output in severely decompensated cardiac patients; the decrease in cerebral perfusion with worsening cardiac decompensation is in all likelihood similarly linked with a decline in cardiac output. The significantly lower mean arterial blood pressure in the group with severe decompensation

Table 3

Cerebral Circulatory and Metabolic Functions in Patients with Severe Congestive Heart Failure

Name	Age	Diagnosis	CBF ml./min./100 Gm. Decomp.	CBF ml./min./100 Gm. Comp.	MABP mm. Hg Decomp.	MABP mm. Hg Comp.	CVR 100 Gm. mm. Hg/ml./min. Decomp.	CVR 100 Gm. mm. Hg/ml./min. Comp.	ml./min./100 Gm. $CMRO_2$ Decomp.	ml./min./100 Gm. $CMRO_2$ Comp.	mg./min./100 Gm. CMR Gluc. Decomp.	mg./min./100 Gm. CMR Gluc. Comp.
L.W.	60	HCVD	42		130		3.10		2.79		2.94	
C.J.	57	AS	34		78		2.29		3.40		3.06	
L.P.	67	AI	40		96		2.40		3.27		6.00	
F.C.	49	MI, AI	42		81		1.93		3.05		3.36	
V.L.P.	46	MS	31		87		2.81		3.16		4.34	
A.B.M.	57	AI	18		83		4.61		1.58		1.44	
J.A.	57	ASHD	39	37	102	104	2.62	2.81	3.56	3.05	3.51	4.44
R.C.	67	ASHD	38		103		2.71		3.53		7.22	
G.H.	57	MS	36		69		1.90		3.80			
J.D.	54	ASHD	50	47	75	82	1.50	1.74	4.70	3.35	4.00	4.70
W.D.	66	ASHD	42		93		2.21		3.23		3.78	
J.F.A.	57	HCVD	62	56	185	181	2.98	3.23	3.19	2.49	4.34	
F.M.	54	ASHD	31	34	112	96	3.61	2.82	2.95	3.57	4.03	4.42
Mean	58		39	44	100	116	2.66	1.22	3.24	3.11	4.00	4.52
S.E.			2.86	5.0	8.48	22.22	0.233	0.318	0.191	0.234	.4252	
			Comparison of pretreatment and posttreatment values (4 patients)									
Mean difference				−2.0		−3.0		−.028		−.485		.673
S.E.				1.87		1.57		0.2545		.1296		.4976
p				>.3		>.1		>.8		<.05		>.8
			Comparison of values with the results in patients with mild to moderate congestive heart failure									
Mean—mild			51		121		2.49		3.8		4.51	
Mean—severe			39		100		2.66		3.24		4.00	
Difference			12		21		0.17		0.56		.51	
p			<.01		<.05		>.6		<.02		>.3	

Abbreviations, see table 1.

may have reflected a more profoundly decreased cardiac output in these patients; however, this difference in blood pressure was due in large part to the greater number of hypertensive subjects in the group with mild to moderate decompensation.

Achievement of optimal compensation was accompanied by a decline in cerebral blood flow in 8 of 15 subjects who were restudied following treatment; the values were essentially unchanged in the remaining 7. Although the mean decline of 8 ml. per 100 Gm. per minute was statistically significant, the data do not clearly establish that relief of the congestive state will be associated in most instances with a decline in cerebral perfusion. Only in patients with mild to moderate decompensation in whom the initial observations were similar to or somewhat greater than "normal" was the decrease of significant proportions. The reason for the decline following treatment is obscure. Although changes in carbon dioxide content following treatment were noted, these were poorly correlated with the magnitude of the change in cerebral blood flow. It is conceivable that in these subjects cerebral blood flow decreased as a result of a contraction of the intravascular volume. It has been shown in this laboratory[13] that selective expansion of the plasma volume results in an increase in cerebral blood flow; moreover, it is increased in hypervolemic subjects with acute glomerulonephritis and returns to normal following recovery.[13] Another possibility is that the therapeutic measures employed exerted an effect on the cerebral circulation similar to that reported with aminophylline in decompensated cardiac patients.[4]

Table 4

Blood Constituents in Patients with Severe Congestive Heart Failure

Name	Arterial O_2 content vol. %		Arteriocerebral venous O_2 diff., vol. %		Arterial CO_2 content, vol. %		Arterial pH		Arterial pCO_2	
	Decomp.	Comp.	Decomp.	Comp.	Decomp.	Comp.	Decomp.	Comp.	Decomp.	Comp.
L.H.	14.84		6.65		49.30		7.32		49	
C.J.	17.08		10.01		44.44		7.45		35	
L.F.	17.00		8.18		45.71		7.36		42	
F.C.	17.06		7.27		46.56		7.41		38	
V.L.P.	21.13		10.20		39.40		7.36		39	
A.B.M.	16.79		8.80		41.09		7.53		27	
J.A.	18.75	17.45	9.14	8.23	43.88	44.69				
R.C.	16.83		9.30		48.73		7.38		44	
G.H.	19.91		10.50		35.19					
J.D.	13.96	17.54	6.69	8.53	44.31	50.77	7.41	7.42	35	43
W.D.	13.25		7.70		42.58		7.43		33	
J.F.A.	13.33	12.57	5.15	4.44	60.77	58.98	7.42	7.40	48	47
F.M.	17.71	19.82	9.50	10.51	45.43	40.89	7.43	7.40	37	37
Mean	16.74	16.84	8.39	7.92	45.18	48.83	7.41	7.41	39	
S.E.	0.668	1.527	0.4482	1.267	1.67	3.94	.0167		1.98	
	Comparison of pretreatment and posttreatment values (4 patients)									
Mean difference		.907		.31		.235		−.01		2.33
S.E.		1.165		.668		2.345		.012		2.68
p		>.4		>.6		>.9		>.4		>.4
	Comparison of values with the results in patients with mild to moderate congestive heart failure									
Mean—mild to moderate	17.27		7.68		44.85		7.41		38	
Mean—severe	16.74		8.39		45.18		7.42		39	
Difference	.53		.71		0.33		.01		1	
p	>.5		>.2		>.7		>.6		>.8	

Otherwise, there is no ready explanation for these changes following treatment.

The results in these 37 decompensated cardiac patients indicate that the status of the cerebral circulation in persons with congestive heart failure is chiefly dependent upon the severity of the congestive state. This finding may resolve some of the existing confusion regarding cerebral hemodynamic alterations in patients with cardiac decompensation.

Conclusions

Cerebral blood flow and metabolism were normal in patients with mild to moderate congestive heart failure, although cerebral vascular resistance was significantly increased.

Cerebral blood flow was significantly reduced in persons with severe congestive heart failure.

Relief of the congestive state was associated with a decline in cerebral blood flow and mean arterial blood pressure in 10 subjects with mild to moderate congestive failure.

Alterations in cerebral blood flow in decompensated cardiac patients are largely dependent upon the severity of the congestive state.

Summario in Interlingua

Le fluxo de sanguine e le metabolismo cerebral esseva normal in patientes con leve o moderate grados de congestive disfallimento cardiac, sed le resistentia cerebro-vascular esseva significativemente augmentate.

Le fluxo de sanguine cerebral esseva significativemente reducite in subjectos con grados sever de congestive disfallimento cardiac.

Alleviamento del stato congestive esseva associate con un declino del fluxo de sanguine cerebral e del tension arterial medie in 10 subjectos con leve o moderate grados de disfallimento cardiac.

Alterationes in le fluxo del sanguine cerebral de patientes a cordes discompensate depende grandemente del grado de severitate del stato congestive.

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. . . Notwithstanding the brilliant sunshine emanating from the discovery of auscultation by Laennec,—a discovery, which, according to M. Bertin, "has, in a few years, more completely illumined the diagnosis of the diseases in question, than all the other modes of exploration had done for two centuries"; the great body of the profession still deny that the piercing ray has reached its destination, still doubt the utility of auscultation in reference to the primary organ of the circulation, still find the ordinary symptoms beset with their accustomed difficulties, still complain, in short, that the obscurity which involves the diseases of which we speak, is scarcely less profound than ever: and, while conflicting opinions are embarrassing the judgment, and undermining the confidence of the patient investigator of truth, there is a general outery for an additional mass of well attested evidence, which may bring the subject to some kind of a conclusion.—J. HOPE, M.D. *Diseases of the Heart and Great Vessels.* London, William Kidd, 1832, p. 11.

Cerebral Hemodynamic and Metabolic Studies in Patients with Congestive Heart Failure

II. Observations in Confused Subjects

By SEYMOUR EISENBERG, M.D., LEONARD MADISON, M.D., AND WILLIS SENSENBACH, M.D.

THE SUPERVENTION of mental aberrations in persons with advanced congestive heart failure often heralds the onset of rapid deterioration and death. The prognostic implications of such manifestations were appreciated more than 50 years ago,[1] but their pathogenesis has remained an enigma. It has been suggested that edema, ischemia, or anoxia of the brain is responsible for these symptoms; digitalis, mercurial diuretics, hypertension, and cerebral arteriosclerosis have also been implicated. Previous studies[2-4] of cerebral flow and metabolism in persons with congestive heart failure have not yielded information germane to this problem, as they apparently included only lucid patients. Moreover, the actual effect of the congestive process on cerebral hemodynamics and metabolism, regardless of mental state, has been the subject of considerable controversy.[2-4]

Recently published data from this laboratory[5] indicate that cerebral blood flow is indeed moderately diminished in alert persons with severe congestive failure. The present study was undertaken to determine whether cerebral perfusion and metabolism were further altered in those cardiac subjects with severe cerebral symptoms.

Materials and Methods

Twenty-four persons with severe, intractable congestive heart failure were studied; this diagnosis was established by the usual clinical criteria of breathlessness, cardiac enlargement, venous distention, and hepatomegaly, and was quite evident in every instance. In addition, these subjects displayed particular features that served to distinguish them from the usual decompensated cardiac patient. First, mental aberrations were prominent in all patients; this varied from simple confusion and somnolence to actual coma. Many of the subjects were slightly icteric and many displayed modest azotemia; however, at postmortem examination little histologic evidence of intrinsic renal or hepatic disease was encountered. Although dyspnea was prominent if the slightest exertion were undertaken, weakness and fatigability had become the dominant symptoms. Twenty-two of the 24 subjects died within several weeks following the onset of this constellation of clinical manifestations. Care was taken to exclude from the study patients with evident cerebral arteriosclerosis and individuals with preexisting renal, hepatic, or mental disease. The possibility of cerebral arteriosclerosis could not be completely excluded on clinical grounds, but none of the subjects had had previous cerebral infarctions; moreover, significant obliterative disease of the cerebral vessels was later excluded in all of the subjects on whom a postmortem examination was performed. Therapy included the usual regimen of digitalis, sodium restriction, and diuretics as required. Electrolyte derangements, particularly hyponatremia, were encountered in several of these patients. In most instances, however, the mental symptoms were present despite a normal serum sodium concentration, and when symptomatic hyponatremia was thought to be present, its correction did not significantly alter the patient's mental state.

Cerebral blood flow was measured by the nitrous oxide method of Kety and Schmidt[6] as modified by Scheinberg and Stead.[7] The analytic methods employed were described in a previous communication.[8] Mean arterial blood pressure was measured directly from a convenient artery by a damped mercury manometer. Cerebral vascular resistance was calculated from the cerebral blood flow and the mean arterial blood pressure and the cerebral me-

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abolic rate of oxygen and glucose from the cerebral blood flow and arteriocerebral venous oxygen and glucose differences. The pH was determined by means of a Cambridge Research Model pH meter, and the carbon dioxide tension was calculated from the monogram of Singer and Hastings.[9]

Six patients were studied during both confused and lucid intervals. Four subjects had been studied prior to the development of mental aberrations, and 2 improved to the point of complete lucidity, at which time they were restudied.

The results in these confused persons with terminal congestive heart failure have been compared with the findings in lucid patients with "mild to moderate" and "severe" congestive heart failure. The results of the cerebral hemodynamic and metabolic studies in these lucid individuals were the subject of a previous report,[5] and the criteria for classifying the congestive state as "mild to moderate" or "severe" were presented in detail. In brief, patients with "mild to moderate" failure responded promptly to digitalis administration and rarely required diuretic measures. Subjects classified as "severe" cardiac subjects had more marked cardiac enlargement, responded slowly to therapy, and usually required intermittent diuretic agents to remain edema-free.

Results

The results of the hemodynamic and metabolic studies in the 24 confused cardiac patients are presented in tables 1 and 2. Mean values for the previously published[5] studies in lucid patients with congestive heart failure are included for purposes of comparison; in 21 of these lucid subjects the congestive state was classified as mild to moderate and in 13 as severe. Six patients were studied during both lucid and confused periods, and the differences were analyzed by the method of paired observations.

The mean cerebral blood flow for the confused patients was 26 ml. per minute per 100 Gm., which represents a profound reduction in cerebral perfusion. This differs significantly ($p < .001$) from the values obtained in mild to moderate (51 ml. per minute per 100 Gm.) and severe but lucid (39 ml. per minute per 100 Gm.) subjects with congestive heart failure. The cerebral vascular resistance was 3.61 mm. Hg per ml. per minute per 100 Gm., which significantly ($p < .01$) exceeded the values in cardiac subjects without mental symptoms. The mean arterial blood pressure was 98 mm. Hg in the confused patients; this did not differ from the mean value in the severe but lucid group but was significantly ($p < .01$) less than the mean pressure of the group with mild to moderate congestive failure. The arteriovenous oxygen difference was 10.36 volumes per cent in the confused patients, which significantly ($p < .001$) exceeded the values obtained in lucid cardiacs. The value for cerebral oxygen consumption of 2.71 ml. per minute per 100 Gm. in the confused subjects was significantly ($p < .01$) less than that obtained in alert individuals with mild to moderate failure but did not differ significantly from the value in lucid patients with severe congestive heart failure. The arterial carbon dioxide content was significantly ($p < .05$) decreased in the confused group as compared with the results in alert subjects; there was, however, no significant difference in the partial pressure of carbon dioxide.

In 6 instances there was an opportunity to study the same individuals during alternating states of mental confusion and alertness. During periods of mental confusion significant changes were encountered. The cerebral blood flow decreased significantly ($p < .01$), and this was associated with an increase in both cerebral vascular resistance ($p < .05$) and mean arterial blood pressure ($p < .05$). The arteriovenous oxygen difference was significantly increased ($p < .001$) during the confused interval, but there was, nevertheless, a significant ($p < .05$) decrease in cerebral oxygen consumption. Arterial carbon dioxide was significantly ($p < .01$) decreased during the "confused" interval.

Discussion

The results of this study indicate that the blood supply to the brain may be profoundly decreased in persons in the terminal stages of congestive heart failure. This is commonly associated with mental aberrations of varying severity, although occasional patients will remain lucid despite this perfusion defect. The clinical impression that the supervention of mental symptoms in chronic congestive heart

Table 1

Cerebral Circulatory and Metabolic Functions in Confused Subjects with Congestive Heart Failure

Name	Diagnosis	Age	CBF ml./min./100 Gm. C*	CBF ml./min./100 Gm. L†	MABP mm. Hg C	MABP mm. Hg L	CVR mm. Hg/ml./min./100 Gm. C	CVR mm. Hg/ml./min./100 Gm. L	$CMRO_2$ ml./min./100 Gm. C	$CMRO_2$ ml./min./100 Gm. L	CMR gluc. mg./min./100 Gm. C	CMR gluc. mg./min./100 Gm. L
E.R.K.	ASHD	65	33		87		2.64		2.94		3.63	
F.K.K.	ASHD	64	24		145		6.04		2.36		2.16	
B.R.B.	ASHD	63	29		78		2.69		2.63		3.48	
G.D.	RHD	43	23		66		2.87		2.87		2.53	
W.D.	ASHD	66	25	42	105	93	4.20	2.21	2.83	3.23		3.78
J.D.	ASHD	63	28		116		4.14		3.12		2.80	
J.A.B.	AI, MS, AS, MI	54	29		90		3.10		3.89		4.35	
W.S.R.	ASHD, AI	79	37		96		2.59		3.29		2.96	
T.P.H.	ASHD	67	24	55	72	81	3.00	1.47	2.52	3.80		
W.E.J.	HCVD	66	23	42	143	128	2.25	3.05	2.25	2.66	2.76	
R.M.	ASHD	56	20		117		5.85		0.88		1.40	
J.L.	ASHD	61	22	32	107	80	4.86	2.50	2.82	3.05	3.52	2.24
G.P.M.	ASHD	61	27		87		3.22		2.11		2.43	
J.T.W.	ASHD	51	27	38	89	69	3.30	1.82	2.70	2.64	4.32	2.66
R.C.	AS, MI	59	23		83		3.61		2.77		3.45	
R.C.Z.	Myocarditis	53	32	48	116	99	3.63	2.06	3.21	4.11	3.20	7.20
G.B.	AS, AI, MS	45	26		81		3.12		2.96		4.68	
M.W.	HCVD	66	32		129		4.03		3.62		2.56	
H.H.K.	ASHD	63	23		108		4.70		2.46			
B.H.J.	MS, AI, AS	53	25		84		3.30		2.50			
M.D.R.	ASHD	60	21		56		2.67		2.30		3.78	
Mean		60	26		98		3.61		2.71		3.18	3.97
S.E.			0.959		5.209		0.227		0.382		.207	
Comparison of values in 6 persons during alternating states of confusion and lucidity (paired observations)												
Mean—lucid			43		92		2.18		3.25		3.97	
Mean—confused			26		106		3.54		2.72		3.18	
Difference			17		14		1.36		0.53		0.79	
p			<.01		<.05		<.05		<.05		>.2	
Comparison of values in confused subjects with values in lucid patients with severe congestive heart failure												
Mean—severe		39	100				2.66		3.24		4.00	
Mean—confused		26	98				3.61		2.71		3.18	
Difference		13	2				0.95		0.53		.82	
p		<.001	>.8				<.01		>.1		>.05	
Comparison of values in confused subjects with the values in lucid patients with mild to moderate congestive heart failure												
Mean—mild to moderate		51	121				2.49		3.80		4.51	
Mean—confused		26	98				3.61		2.71		3.18	
Difference		25	23				1.12		1.09		1.33	
p		<.001	<.01				<.001		<.01		<.01	

*C, confused.

†L, lucid.

AI, aortic insufficiency; AS, aortic stenosis; ASHD, arteriosclerotic heart disease; HCVD, hypertensive cardiovascular disease; MI, mitral insufficiency; MS, mitral stenosis; RHD, rheumatic heart disease.

failure constitutes a poor prognostic sign is amply supported by the extremely high mortality among the patients comprising this study.

The clinical manifestations of passive congestion in the patients with mental symptoms were qualitatively and quantitatively similar to those in lucid persons with severe conges-

Table 2

Blood Constituents in Confused Subjects with Congestive Heart Failure

Name	Arterial O_2 content vol. % C*	L†	Arteriocerebral venous O_2 diff., vol. % C	L	Arterial CO_2 content, vol. % C	L	Arterial pH C	L	Arterial pCO_2 C	L
E.R.K.	17.27		8.90		45.39		7.37		43	
C.K.K.	13.98		9.84		47.76		7.40		43	
E.R.B.	16.54		9.08		45.49		7.45		37	
C.D.	16.28		12.48		30.11		7.46		24	
W.D.	15.92	13.25	11.31	7.70	38.70	42.58	7.43			33
J.D.	17.90		11.14		46.01		7.41		39	
J.A.B.	19.98		13.43		38.33		7.44		32	
W.S.R.	15.40		8.88		43.25		7.43		35	
T.P.H.	15.51	14.63	10.51	7.40	46.04	55.93				
W.E.J.	17.02	12.64	9.80	6.33	46.40	54.14	7.37	7.25	42	61
R.M.	17.52		4.42		38.54		7.45		30	
J.L.	18.00	16.91	12.83	9.52	46.23	52.01	7.43	7.38	38	
G.P.M.	15.16		7.82		21.82		7.35		21	
J.T.W.	17.97	15.37	10.58	6.95	46.28	57.50	7.60	7.51	28	28
R.C.	16.26		12.06		33.52		7.47		25	
R.C.Z.	14.82	18.07	10.03	8.57	27.83	43.67		7.38		40
G.B.	17.16		11.37		41.19		7.44		35	
M.W.	15.64		11.31		49.38		7.42		34	
H.H.K.	16.25		10.70		31.52					
B.H.J.	14.96		10.16		37.25					
M.D.R.	16.17		10.94		41.03		7.50		30	
Mean	16.46		10.36		40.10		7.44	7.38	34	
S.E.	0.30		0.42		1.63		.0146		1.70	
Comparison of values in 6 persons during alternating states of confusion and lucidity (paired observations)										
Mean—lucid		15.14		7.74		50.97		7.38		
Mean—confused		16.54		10.84		41.91		7.47		
Difference		1.40		3.10		9.06		.09		
p		>.2		<.001		<.01		<.05		
Comparison of values in confused subjects with values in lucid patients with severe congestive heart failure										
Mean—severe	16.74		8.39		45.18		7.41		39	
Mean—confused	16.46		10.36		40.10		7.44		38	
Difference	.28		1.97		5.08		.03		1	
p	>.4		<.01		<.05		>.1		>.8	
Comparison of values in confused subjects with the values in lucid patients with mild to moderate congestive heart failure										
Mean—mild to moderate	17.27		7.68		44.85		7.42		38	
Mean—confused	16.46		10.36		40.10		7.44		34	
Difference	.81		2.68		4.75		.02		4	
p	>.1		<.001		<.02		>.3		>.05	

*C, confused.
†L, lucid.

tive heart failure, and it is extremely doubtful that the reduction in cerebral blood flow was related to impedance imposed by an increase in the severity of cerebral congestion. It is more likely that the sharp decline in cerebral perfusion associated with the development of mental symptoms in these subjects resulted from hemodynamic alterations in the arterial segment of the vascular tree. It was suggested in a previous publication[5] that the

decline in cerebral blood flow in alert persons with severe cardiac decompensation was related to a decrease in cardiac output, which is consonant with the established relationship of renal[10] and hepatic blood flow[11] to cardiac output in persons with chronic congestive heart failure. It is therefore suggested that in the confused cardiac patients herein reported cardiac output had declined to critical levels and that as a result cerebral perfusion was impaired to the point that functional abnormalities supervened. There was no reduction in mean arterial blood pressure to support such a formulation; all of these patients, however, ultimately developed severe hypotension with clinical evidence of circulatory collapse. This was thought to represent a further fall in cardiac output or true "cardiac shock."

The failure of the brain to function adequately in these persons with "end-stage" congestive failure could have been due to abnormalities other than the poor blood supply to the brain. Alterations in electrolyte concentration, oxygen content, or pH of the arterial blood of sufficient magnitude to account for the mental symptoms, however, were not encountered. Therefore, it is likely that the failure of cerebral function under these circumstances was the result of a severe metabolic abnormality intimately linked with the critical reduction in the blood supply to the brain. These data, however, do not define precisely the nature of this abnormality. Although cerebral metabolic rate for oxygen was decreased in most of these confused cardiac patients, the results do not clearly establish that the mental symptoms resulted from inadequate utilization of oxygen. It is conceivable, however, that the estimation of total cerebral metabolic rate for oxygen fails to reflect abnormalities in individual cells or areas of the brain and that distributional changes could occur that might not be perceived by the method employed; under circumstances where perfusion is inadequate and oxygen is in critical supply, those centers concerned with consciousness could deteriorate while the remainder of the brain is functioning adequately.

This study supports the concept that cerebral ischemia is chiefly responsible for the mental symptoms encountered in patients with far-advanced congestive heart failure. There was little evidence in these 24 cardiac patients that factors such as electrolyte abnormalities, digitalis therapy, anoxemia, or cerebral arteriosclerosis contributed significantly to the development of their altered mental state.

Conclusions

Cerebral blood flow was profoundly diminished (26 ml. per minute per 100 Gm.) in 24 mentally confused persons with advanced congestive heart failure.

Cerebral utilization of oxygen was decreased in the majority of these confused individuals.

The supervention of mental symptoms in patients with advanced congestive heart failure has grave prognostic implications.

Acknowledgment

This work was done with the technical assistance of Miss Mary Frances Camp and Miss Mildred T. Brennan.

Summario in Interlingua

Le fluxo de sanguine cerebral esseva marcatemente reducite (26 ml per minuta per 100 g) in 24 mentalmente confuse subjectos con avantiate formas de congestive disfallimento cardiac.

Le utilisation cerebral de oxygeno esseva reducite in le majoritate de iste confuse individuos.

Le supervention de symptomas mental in patientes con avantiate disfallimento congestive del corde ha un grave signification ab le puncto de vista del prognose.

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I believe it was Samuel Butler who said that one of the arts of living consists in the ability to come to adequate conclusions from inadequate evidence. In any event, much of our practical living involves action on the basis of assumptions of varying degrees of probability. We often forget, only to recall at leisure later, that the most improbable events are not impossible. They are only extremely improbable. In just this sense, I would think it highly improbable that medical students can wisely be expected to acquire for the first time at so late a stage in their preparation, basic intellectual honesty, industrious habits, dependable morality, or freedom from the shackles of tradition and authoritarianism if in neither home nor secondary school they have met examples of or training in honesty, responsibility, hard work, and freedom of mind and spirit. I do not think we often stop to realize how much of these qualities we assume to be present in medical students, nor how much we rely, unconsciously, on these qualities that are essential to the effectiveness of our institutions of higher learning.—ALAN GREGG, M.D. *Challenges to Contemporary Medicine.* New York, Columbia University Press, 1956, p. 114.

Idiopathic Dilatation of the Pulmonary Artery

By MADHUKAR DESHMUKH, B.S.M.B.B.S., SULAHATTIN GUVENC, M.D., LAMBERTO BENTIVOGLIO, M.D., AND HARRY GOLDBERG, M.D.

IDIOPATHIC dilatation of the pulmonary artery has been recognized as a clinical entity since 1923, when Wessler and Jaches described the first case.[1] Since dilatation of the pulmonary artery is frequent in many congenital as well as acquired heart lesions, differentiation of this benign anomaly from the latter group is of prime importance. With advances in knowledge of congenital heart disease and improvement in the diagnostic methods, antemortem recognition of this condition has become possible. This report of 13 patients, in whom heart catheterization was performed, aims at drawing attention to this anomaly and elaborating its clinical and physiologic aspects.

Material

The 13 patients of this series were referred to the Bailey Thoracic Clinic for evaluation of their cardiac status. Eight were male and 5 were female, and their ages ranged from 3 to 35 years. All these patients had complete clinical examinations including history, physical examination, complete blood count, Kolmer's serologic reaction, and other pertinent laboratory studies. Roentgen examination of the heart was performed in the 4 conventional positions. Every patient had a 12-lead electrocardiogram and, in some, leads V_{3R} and V_{4R} were recorded. Right-sided heart catheterization was performed in the normal way. A systolic pressure of 30 mm. Hg was regarded as the limit of normal in the right ventricle.[2]

Results

All but one of the patients were referred for cardiac evaluation because of the incidental discovery of a heart murmur during routine physical examinations. In 1 patient the abnormality was noted for the first time in a routine roentgenogram of the chest. The heart murmur in this patient was so faint that it escaped attention. Absence or mildness of symptoms was the prominent finding in this series. Five of the patients complained of mild fatigue and 2 of dyspnea of mild degree on exertion; 1 had pain in the chest and 3 had palpitation.

The abnormal physical findings were limited to cardiac palpation and auscultation. A systolic murmur was heard in the left second and third interspaces, and the pulmonic second sound was accentuated. The character and the intensity of this systolic murmur varied from patient to patient. In 3 patients a systolic thrill was felt where the murmur was loudest, i.e., the second and third left intercostal space. In addition, in 5 patients splitting of the first heart sound was heard at the apex and was interpreted as a systolic ejection click (fig. 1). In 1 patient an early diastolic murmur, blowing in character, was heard in the pulmonary area.

The laboratory studies were within normal limits. Kolmer's serologic reaction was negative in all.

The roentgen examination showed a normal cardiac silhouette and, in striking contrast, dilatation and prominence of the pulmonary artery segment in posteroanterior and right anterior oblique positions (figs. 2 to 4). In one case the dilatation extended to the left and right main branches (fig. 3). The peripheral lung fields were of normal vascularity in each instance, and there was no chamber enlargement. The electrocardiogram was normal in all instances.

Cardiac catheterization showed no evidence of left-to-right shunt. The right ventricular and pulmonary artery pressures were within normal limits. A small systolic pressure gradient was noted across the pulmonic orifice in some cases. The cardiac output was normal. The peripheral oxygen saturation was normal except in patients 4, 5, and 8. In these patients the catheterization was performed under

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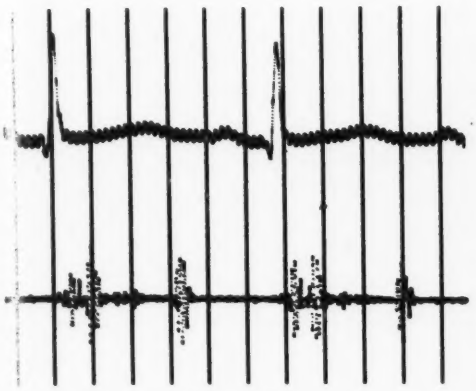

Figure 1

Electrocardiogram and phonocardiogram showing a systolic ejection click.

general anesthesia, and the slight peripheral unsaturation was attributed to diminished pulmonary ventilation (table 1).

Discussion

In the patient described by Wessler and Jaches, the diagnosis of idiopathic dilatation of the pulmonary artery was made on the basis of roentgenographic findings.[1] Oppenheimer in 1933 described a series of 8 patients having this condition.[3] Two of these had postmortem examination and the main finding was dilatation of the pulmonary artery without any other congenital defect or malformation. The pulmonary vasculature revealed intimal thickening. In the other 6 patients the diagnosis was made on the basis of clinical and roentgenologic similarity with these 2. All the patients in the group had severe symptoms. All but 1 of them had shortness of breath; 5 had cyanosis, and 4 had evidence of congestive failure. In this respect the above group of patients differs markedly from subsequent reports[4–6] in which the patients had few or no symptoms. It is difficult, therefore, to maintain the diagnosis of idiopathic dilatation of the pulmonary artery in his patients. It is probable that they had some other disease, such as primary pulmonary hypertension with secondary dilatation of the pulmonary artery.

Kourilsky and his associates[7] presented 10 cases of this condition, 9 with postmortem examination. Only 6 cases can be accepted as having idiopathic dilatation of the pulmonary artery because 2 had atrial septal defect and the third had mitral stenosis.

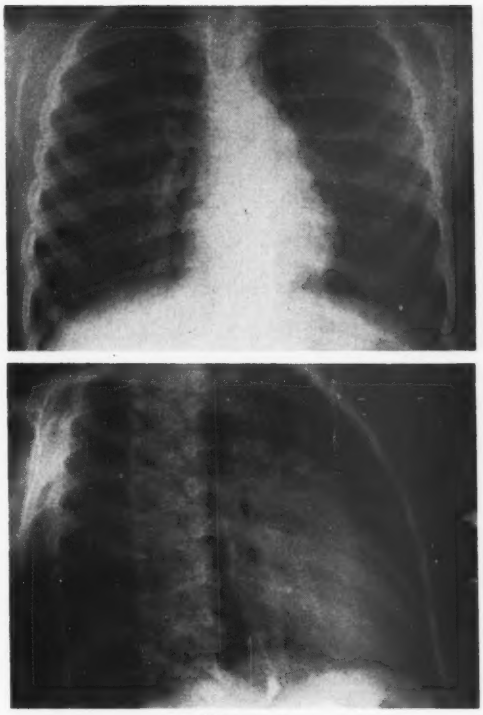

Figure 2

Case 4. Roentgenogram of the heart in postero-anterior (top) *and right anterior oblique* (bottom) *positions, showing dilatation of pulmonary artery and normal heart size.*

Gold[8] stressed the presence of a hypoplastic aorta in the diagnosis of idiopathic dilatation of the pulmonary artery. In the absence of the hypoplastic aorta, he considered the dilatation of the pulmonary artery to be due to Ayerza's disease or primary pulmonary sclerosis. The deformity was thought to result from unequal division of the truncus arteriosus communis.[7, 8] Hypoplasia of the aorta, however, is an inconstant finding,[7] and its estimation in clinical practice by routine roentgenologic examination is far from satisfactory.

Differentiation of idiopathic dilatation of the pulmonary artery from other congenital and acquired lesions with dilated pulmonary

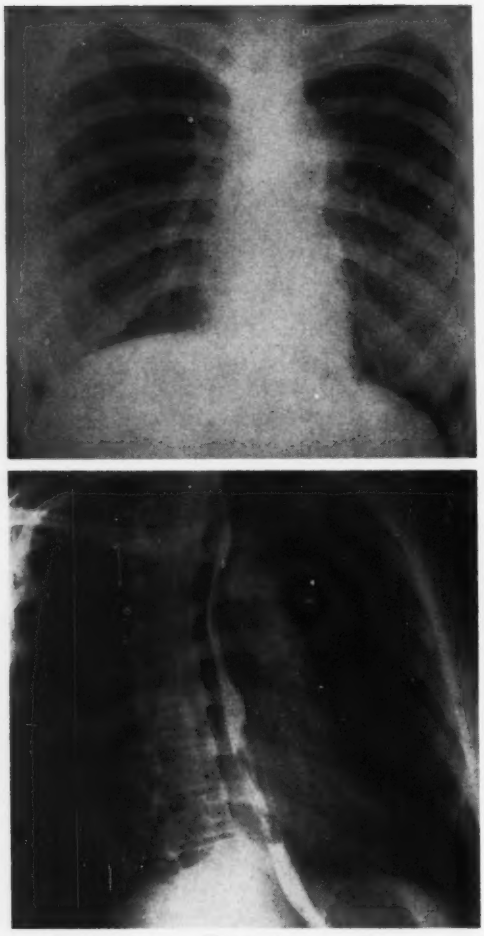

Figure 3

Case 7. Roentgenograms of the heart showing dilatation of the main pulmonary artery segment and its extension to the main branches.

artery is of primary importance because most of the latter abnormalities can be corrected by surgical procedures (table 2). Idiopathic dilatation of the pulmonary artery is on the other hand a benign anomaly and does not require surgical intervention. Clinically, an important diagnostic lead is absence or mildness of symptoms.[4-6] Careful history taking in some of our symptomatic patients suggested anxiety of iatrogenic origin as the cause of their symptoms rather than the presence of cardiac disease. Severe restrictions were

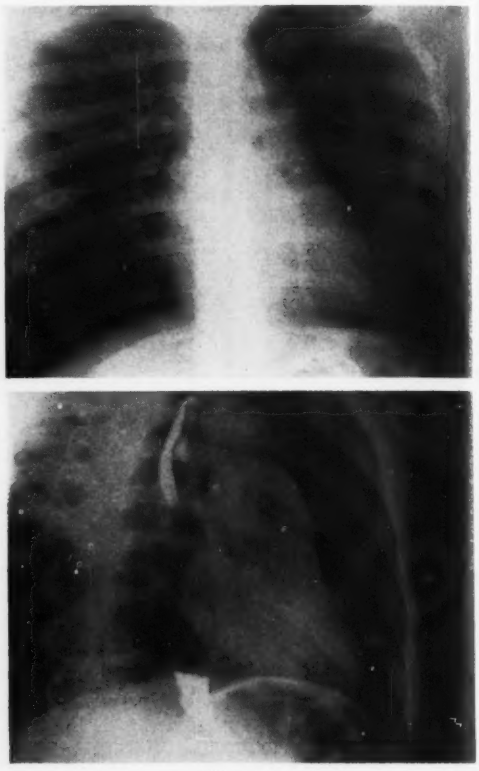

Figure 4

Case 10. Roentgenograms of the heart showing more severe dilatation of the pulmonary artery.

imposed because of the murmurs. In fact, 1 man was rejected from the military service because of the murmur, and 1 woman was advised not to have children.

The systolic murmur, which is the most prominent physical finding in this condition, is quite inconstant in character and intensity.[5, 6] In some cases the murmur is quite rough and resembles that of pulmonary stenosis, whereas in others it is blowing in character, giving the auscultatory impression of an atrial septal defect. The systolic ejection click, which we heard in 5 patients, is of no diagnostic aid because it may be present in other conditions in which there is dilatation of the great vessels (fig. 1).[9]

Roentgen examination of the heart is important, particularly for comparison of the

Table 1

Hemodynamic Data

Case	PCV	Pressure (mm. Hg) PA	RV	RA	BA	Oxygen content of blood (vol. %) SVC	IVC	RA	RV	PA	BA	Oxygen capacity (vol. %)	Systemic oxygen saturation (%)	Oxygen consumption (ml./min.)	Cardiac output (L./min.)
1	(3)†	10/5 —(7)	25/2	(3)	112/56 —(76)	12.4	14.5	13.3	12.2	12.5	16.6	18.2	91.2	133.8	4.3
2	—	14/2 —(8)	14/0	(2)	132/76 —(98)	12.9	15.2	13.3	13.5	13.5	19.8	20.3	97.2	265.7	5.3
3*	(9)	21/10 —(17)	21/1	(3)	125/69 —(85)	14.0	15.6	13.2	13.6	14.5	17	17.8	95.5	220	7.9
4*	—	20/4	20/0	(0)	100/60 —(78)	10.6	9.3	10.4	10.6	10.9	13.9	15.4	90.3	—	—
5*	(3)	12/5 —(8)	18/0	(0)	90/50 —(65)	11.6	—	12.2	12.5	13	14.9	17.4	86	—	—
6*	(6)	11/5 —(9)	19/4	(3)	96/58 —(71)	13.4	11.1	13.1	12.7	12.9	16.3	17.1	95.3	—	—
7	(2)	13/5 —(7)	18/4	(1)	105/69 —(82)	15.9	—	14.7	15	14.8	20.2	21.8	93	299	5.5
8*	—	25/8	25/0	(4)	80/40 —(64)	11.6	13.2	12.2	12.2	11.5	15.4	17	90.6	88.1	2.2
9	(4)	12/4	20/3	(2)	136/60 —(84)	13.3	11.2	12.2	11.8	11.9	16	17.2	93	194	4.7
10*	(5)	24/12	25/0	(2)	—	15.9	14.5	15.6	15.8	15.8	19.1	20.9	91.4	161.9	4.9
11*	—	22/15 —(18)	22/0	(2)	130/85 —(100)	14.5	16.3	11.6	14.8	15.3	18.6	19.7	94	—	—
12	(5)	18/4 —(10)	22/2	(1)	140/72 —(91)	13.2	—	12.5	10.9	13.6	18.3	19.6	93.4	—	—
13*	—	20/10 —(14)	25/3	(1)	105/80 —(89)	11.2	—	11.7	11.5	11.1	16.3	18.3	92	—	—

*Catheterization performed under general anesthesia.

†Figures in parentheses represent mean pressures.

BA, brachial artery; IVC, inferior vena cava; PA, pulmonary artery; PCV, pulmonary capillary venous; RA, right atrium; RV, right ventricle; SVC, superior vena cava.

size of the pulmonary artery with the total heart size and with the peripheral pulmonary arteries. The paradox of enlarged main pulmonary artery and diminished vascularity of the periphery of the lung fields, so characteristic of pulmonary stenosis, is absent in this condition. The hyperemic lung fields and the hilar dance, which are common in large left-to-right shunts, are also lacking. The heart size is normal; care should be taken, however, because the prominent pulmonary artery segment may give a false impression of cardiac enlargement. The cardiothoracic ratio is within normal limits.

Electrocardiography is very useful in the differential diagnosis. The patterns of so-

Table 2

Conditions Accompanied by Dilatation of the Pulmonary Artery

Congenital
A. *With intracardiac or extracardiac shunts*
Atrial septal defect
Anomalous pulmonary venous drainage (partial or total)
Ventricular septal defect
Patent ductus arteriosus
Aortopulmonary septal defect
Aortic-right heart fistula
B. *Without shunts*
Pulmonary stenosis (poststenotic dilatation)
Coarctation of the pulmonary artery or its branches
Pulmonary vein stenosis
Idiopathic dilatation of pulmonary artery

Acquired
A. *Cardiac diseases*
Mitral valve disease
Left atrial tumors
Chronic left ventricular failure
B. *Pulmonary diseases*
1. Parenchymal
Pulmonary agenesis, cysts, extensive pulmonary emphysema, and fibrosis, granulomas (sarcoid, berylliosis, miliary tuberculosis), carcinoma of the lung, and Hamman-Rich syndrome.
2. Vascular
Primary pulmonary hypertension
Secondary pulmonary hypertension due to repeated pulmonary emboli, collagen diseases, schistosomiasis.
Syphilis
C. *Hyperkinetic circulatory states*
Anemia
Hyperthyroidism
Paget's disease
Beriberi
Systemic A-V fistulas

called diastolic and systolic overload of the right ventricle found, respectively, in atrial septal defect and pulmonary stenosis are not present. The pattern of diastolic overload of the left ventricle commonly found in interventricular septal defect and patent ductus arteriosus is also absent. The electrocardiogram in this condition is normal; if significant abnormality is present, other types of cardiac disease should be suspected.

In spite of the well-marked clinical, radiologic, and electrocardiographic features, the diagnosis of idiopathic dilatation of the pulmonary artery is one of exclusion. We think that cardiac catheterization is superior to angiocardiography as a diagnostic tool. Absence of left-to-right shunt, normal pressure relationships in the cardiac chambers, and normal oxygen saturation are the important findings. A small pressure gradient between right ventricle and pulmonary artery during the systolic ejection phase may exist (fig. 5). Chisholm advocated the concept of trigonoidation of the pulmonary orifice as the mechanism for the basal systolic murmur and the relative stenosis of the pulmonary orifice in simple dilatation of the pulmonary artery.[10] Since the pressure difference in idiopathic dilatation of the pulmonary artery is present without right ventricular hypertension, its probable cause, in our opinion, is the deceleration of blood flow in the dilated pulmonary artery, rather than true anatomic obstruction of the pulmonary valve. This is quite apparent from the hemodynamic expression: resistance = pressure/flow. Flow being constant, pressure varies directly with resistance. Resistance in its turn is related inversely to the radius of the vessel as expressed in the formula $R = \frac{8LV}{\pi r^4}$, in which L and r are the length and the radius of the tube and V is the viscosity of the fluid. In idiopathic dilatation of the pulmonary artery, resistance to blood flow in the pulmonary artery is diminished because of the dilatation of the lumen; consequently there is a proportional drop in pressure, which accounts for the pressure gradient between the right ventricle and the pulmonary artery during systole.

Greene and associates reviewed the literature on idiopathic dilatation of the pulmonary artery and established the following pathologic criteria for its diagnosis: (1) simple dilatation of the pulmonary trunk with or without involvement of the rest of arterial tree; (2) absence of intracardiac or extracardiac shunts; (3) absence of chronic cardiac or pulmonary disease; (4) absence of arterial disease, such as syphilis, or more than minimal atheromatosis or arteriosclerosis of the pulmonary vascular tree.[11] All these criteria, except atheromatosis of the pulmonary vasculature, can be evaluated clinically and by routine laboratory tests, cardiac catheterization, and pulmonary function studies. All the

patients in our group fulfilled these clinical criteria. In addition all had normal hemodynamic findings. In our opinion, the normal pressure in the right ventricle and pulmonary artery should be introduced as the fifth criterion in the diagnosis. The presence of even a mild degree of right ventricular hypertension associated with a systolic gradient across the pulmonic valve should be considered as sufficient evidence for the diagnosis of pulmonary stenosis.

Nothing is known about the etiology of this lesion and relatively little about its pathogenesis and natural history. Assman[12] postulated an unequal division of the truncus arteriosus communis as the possible mechanism, and Kourilsky et al.,[7] Laubry and associates,[13] and Gold[8] supported this idea because of the presence of a hypoplastic aorta in some of their cases. Kaplan and others[5] thought the anomaly represented one aspect of the maldevelopment of the entire pulmonary tree and of congenital weakness of the arterial wall. Our series does not contribute to this issue.

Prognostic evaluation of idiopathic dilatation of the pulmonary artery is limited by the number of clinically studied cases and the lack of adequate follow-up. In the only acceptable autopsied series, however, the 6 proved cases died of unrelated diseases at the ages of 56, 63, 78, 82, 88, and 92. It is therefore believed that idiopathic dilatation of the pulmonary artery is a benign congenital malformation.

Summary

Thirteen patients with idiopathic dilatation of the pulmonary artery are described and their clinical, roentgenologic, electrocardiographic, and hemodynamic features are elaborated. Absence or mildness of symptoms was the most significant finding in this series. A pulmonic systolic murmur was constantly present. The roentgenogram showed various degrees of dilatation of the pulmonary artery in the presence of normal heart size. The electrocardiogram was normal. The hemodynamic studies showed a mild systolic pressure gradient across the pulmonic valve in some cases in the presence of normal right ventricular pressure. A possible explanation of this finding is discussed. Idiopathic dilatation of the pulmonary artery is a benign lesion and does not affect cardiac function to any appreciable degree.

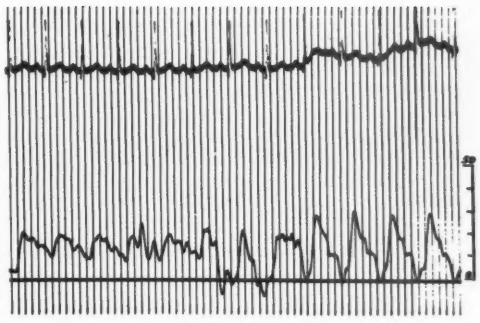

Figure 5

Pressure tracing showing a small systolic ejection pressure gradient between the right ventricle and the pulmonary artery.

Summario in Interlingua

Es presentate 13 casos de idiopathic dilatation pulmono-arterial, e le observationes clinic, roentgenologic, electrocardiographic, e hemodynamic es describite detaliatemente. Le absentia de symptomas o le presentia de solmente leve symptomas esseva le plus significative constatation in iste serie. Un murmure pulmono-systolic esseva constantemente presente. Le roentgenogramma monstrava varie grados de dilatation del alteria pulmonar in le presentia de normal dimensiones cardiac. Le electrocardiogramma esseva normal. Le studios hemodynamic revelava un leve gradiente de pression systolic a transverso le valvula pulmonic in certe casos in le presentia de normal pressiones dextero-ventricular. Un explication possibile de iste observation es offerite e discutite. Dilatation idiopathic del arteria pulmonar es un lesion benigne e non affice le function del corde a grados notabile.

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Connor, A. C., McFadden, J. F., Houston, B. J., and Finn, J. L.: Familial Congenital Complete Heart Block. Am. J. Obst. & Gynec. 78: 75 (July), 1959.

A case of congenital complete heart block is described, and the literature reviewed. A brother had also presented bradycardia and had bouts of cyanosis with Stokes-Adams episodes. The incidence, etiology, symptomatology, and prognosis of this condition are reviewed. Other congenital anomalies are usually found, the most common being an intraventricular septal defect. If congenital complete heart block is the only lesion, the prognosis is good and there is usually no limitation on the patient's activities. Even pregnancy and high-altitude flying have not been contraindicated in reported cases. In this patient, the diagnosis was suspected prenatally because of the slow, regular fetal heart rate (64 to 68 per minute). This condition can be confused with fetal distress and be the cause of unnecessary interference with pregnancy or labor. The criteria for the diagnosis of congenital complete heart block are (1) a slow pulse at an early age, (2) proof by graphic methods, (3) other signs of congenital heart disease, and (4) absence of a history of infection, such as diphtheria or rheumatic fever. The pulse rate is usually from 40 to 60 and may increase with exercise or after atropine or epinephrine. The stroke volume is greater than normal but the cardiac output remains normal.

MAXWELL

Permutation Trial of Diuretics

Chlorothiazide and Hydroflumethiazide

By FREDRIK KIIL, DR. MED.

THE LARGE number of oral diuretics brought on the market every year has increased the demand for a simple screening test by which clinical comparisons can be made between well-known and new diuretics without too large expenditure of time and money. Although all oral diuretics in use probably act through inhibiting the tubular reabsorption of electrolytes and water, it has been apparent, especially since the introduction of derivatives of chlorothiazide, that even small changes in side chains may profoundly alter the pattern of water and electrolyte excretion.

The main purpose of an acute screening test is to compare the potencies of the drugs to excrete sodium and water and to determine whether the new compound may give rise to smaller or larger electrolyte disturbances. The main disturbances that may be produced by chlorothiazide are hypokalemia, hyponatremia, and hypochloremic alkalosis. The hypokalemic effect may be evaluated through the surplus of potassium excreted. The hyponatremic effect is dependent on the ratio between the additional amounts of sodium and water excreted. The hypochloremic effect depends on the magnitude of the ratio between sodium and chloride excretion.

The necessary information about potency and side effects should be attained in as few days as possible. Since the purpose of a diuretic usually is to remove edema caused by heart failure, it is obviously more reasonable to test the drug when it removes edema than when the patient is in sodium balance.

The increase in sodium and water excretion caused by chlorothiazide and derivatives lasts less than 12 hours.[1-3] It should therefore be completely safe with such short-acting diuretics to administer a new one (or a new dose) in a 24-hour interval.

The main purpose of the present investigation is to show that in a properly planned experiment the necessary information concerning acute effects of 2 diuretic agents may be obtained from only 12 patients in 3 days.

Methods

The 12 patients with signs of right heart failure had stayed in the hospital for several days when they were included in this study, but they still had moderate peripheral edema. During the study the patients were on a metabolic ward. They did not receive diuretic agents, but other medications required for control of heart failure, such as digitalis, were continued throughout the trial. None of these medications was initiated during or immediately before the study.

The diet given during and at least 5 days preceding the study was the ordinary low-salt diet used in the hospital for the treatment of heart failure. No attempts were made to define or standardize this diet any further. The first day of the study the patients were allowed to choose how much they wanted to drink. This amount of fluid intake was kept constant during the 2 following days. Four patients with larger degrees of edema were kept in bed, while the others were ambulatory.

Urine was collected in 24-hour periods. The volume was measured to the nearest 5 ml. The urine was analyzed for sodium and potassium concentration by means of a Baird indirect flame photometer. Magnesium was determined by the method of Orange and Rhein,[4] calcium by a modification of the method of Fales,[5] and chloride by the method of Schales and Schales.[6]

The effects of 2 diuretic agents, chlorothiazide and hydroflumethiazide (Rontyl) and placebo tablets (milk sugar) were compared. The difference between hydrochlorothiazide and hydroflumethiazide (6-trifluormethyl-7-sulfamyl-3, 4-dihydro-1,2,4, benzothiadiazin-1, 1-dioxide) is that a chlorine atom is substituted with trifluormethyl.

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Table 1

Sequence of Administration of Drugs and Placebo Tablets

Patients	Days 1	2	3
1	P	R	C
2	P	C	R
3	R	P	C
4	R	C	P
5	C	P	R
6	C	R	P

Treatments: C, chlotride; P, placebo; R, Rontyl.

Table 2

Excretion of Electrolytes and Water

Sequence of drugs	Days 1	2	3	Sum
Sodium (mEq./24 hrs.)				
CPR	162	54	102	318
CRP	146	120	18	284
PRC	87	218	203	508
PCR	126	296	161	583
RPC	138	32	105	275
RCP	296	252	98	646
Sum	955	972	687	2614
Sum C: 1164	Sum P: 415		Sum R: 1035	
Mean C: 194.0	Mean P: 69.2		Mean R: 172.5	
Potassium (mEq./24 hrs.)				
CPR	67	56	64	187
CRP	55	58	37	150
PRC	42	60	67	169
PCR	48	65	57	170
RPC	37	34	59	130
RCP	58	70	47	175
Sum	307	343	331	981
Sum C: 383	Sum R: 334		Sum P: 264	
Mean C: 63.8	Mean R: 55.7		Mean P: 44.0	
Chloride (mEq./24 hrs.)				
CPR	146	61	117	324
CRP	64	111	13	188
PRC	36	185	149	370
PCR	111	237	178	526
RPC	173	44	87	304
RCP	305	194	93	592
Sum	835	832	637	2304
Sum C: 877	Sum P: 358		Sum R: 1069	
Mean C: 146.2	Mean P: 59.7		Mean R: 178.2	
Water (ml./24 hrs.)				
CPR	1820	1130	1325	4275
CRP	895	725	620	2240
PRC	1560	2170	2500	6230
PCR	1310	2265	1475	5050
RPC	1210	720	1255	3185
RCP	1600	2285	1420	5305
Sum	8395	9295	8595	26285
Sum C: 11020	Sum P: 6760		Sum R: 8505	
Mean C: 1835	Mean P: 1125		Mean R: 1420	

The daily doses of the diuretic agents were 2 Gm. of chlorothiazide or 200 mg. of hydroflumethiazide, divided in equal doses given at 8:00 A.M. and 12 noon. Previous investigations[1, 7] had shown these doses to give optimal natriuretic effect. The 2 diuretic agents and placebos were administered in the course of the 3 days of the study in all permutations (table 1). Each sequence of administration was followed for 2 patients, chosen at random, so that on the first 3 days of the study 4 patients received chlorothiazide, 4 hydroflumethiazide, and 4 placebo tablets. Through this design each drug was given the same change in sequence to compare its acute effects.

Results

Table 2 shows the daily excretion of sodium, potassium, chloride measured in milliequivalents and the daily excretion of water measured in milliliters. The sequence of treatments is indicated with letters, so that, for instance, CPR indicates that chlorothiazide was given the first day, placebo the second day, and hydroflumethiazide (Rontyl) the third day. Each value is the average of the daily excretion of 2 patients. The variation from one permutation to another is, however, considerable as shown in the last column of the table. At the bottom of each section of the table the total and the daily mean output of solutes and water during each treatment is given. Slightly more sodium was excreted during chlorothiazide treatment, whereas more chloride was excreted following administration of hydroflumethiazide.

Figure 1 shows that magnesium excretion was independent of urinary flow when the diuresis varied between 600 and 2,500 ml. It is also apparent that the 2 diuretic agents did not specifically influence the excretion of magnesium. Figure 2 shows that calcium excretion varied slightly more than magnesium excretion. There is no correlation between magnesium and calcium output and no evidence that the diuretic agents influenced calcium excretion.

The urine became more alkaline in all 12 cases during chlorothiazide than during placebo administration. The range of the differences in pH was 1.1 to 0.2, with a mean

of 0.67. Between days of hydroflumethiazide and days of placebo administration there was no significant change of pH of urine.

Statistical Treatment

Because of the design of the experiment the error variance could be considerably reduced through analysis of variance. By this technic the variation due to days and to permutations could be accounted for. As indicated in table 3 the variation between permutations and the variation between days are mostly highly significant when tested by comparison with the error mean square. This signifies that by taking into account these sources of variation the error variance is reduced. Since the effects of treatments eventually are demonstrated by comparison with the error variance, the design of the experiment has made it more sensitive and increased the possibility of demonstrating differences between treatments. The effect of treatments may be resolved in 2 comparisons by use of the rules of orthogonality.[8] The first comparison is between the effects of the 2 diuretics. The second comparison is between the diuretics on the one hand and the placebo "treatment" on the other.

The results of these comparisons are shown in the lower parts of table 3. The natriuretic and chloruretic effects of the 2 diuretics are not different as shown by this analysis, whereas the natriuretic and chloruretic effects of both diuretics are far above the placebo effect. Both diuretics increase the diuresis, but water excretion is significantly larger during administration of chlorothiazide than during hydroflumethiazide.

The kaliuretic effect is significantly less for hydroflumethiazide than for chlorothiazide although the difference in potassium excretion between treatments is slight. The hypochloremic effect was tested by comparing the natriuretic and the chloruretic properties of the drugs. If there were no difference between the diuretic agents, the ratio of the excreted amounts of sodium and chloride during hydroflumethiazide and chlorothiazide administration, respectively, should be equal, and accordingly $Na_R/Cl_R \div Na_C/Cl_C = 1$. The sodium:chloride ratio for hydroflumethiazide in all 12 cases was lower than the sodium:chloride ratio for chlorothiazide, and all the ratios as indicated above were below 1, the range being 0.89 to 0.61, with a mean of 0.74. The probability that this finding should be due to chance is ½ to the twelfth power or less than 1 per 1,000. At equal natriuretic effect hydroflumethiazide accordingly has a higher chloruretic effect than chlorothiazide.

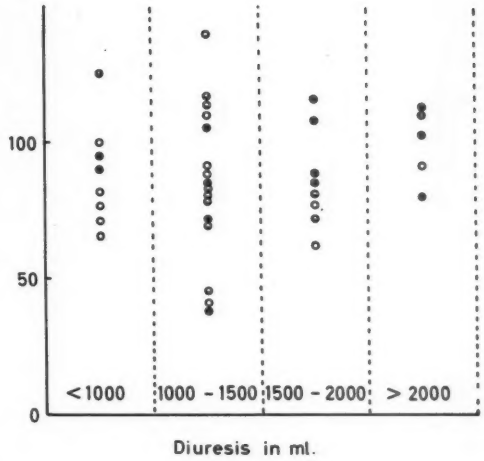

Figure 1

Magnesium excretion, mg./24 hours, (ordinate) *at various urinary flows during permutation trial. Solid circle, chlorothiazide (Chlotride); half-solid circle, hydroflumethiazide (Rontyl); open circle, placebo tablets.*

When a diuretic agent increases the sodium concentration of urine above normal, through prolonged use it may reduce the sodium concentration of the extracellular fluid and accordingly have a hyponatremic effect, especially if the sodium concentration of the additionally excreted urine is far above the plasma sodium level.

If Na_R and Na_P are the sodium excretions during hydroflumethiazide and placebo administration, respectively, and D_R and D_P are the diureses during these treatments, the expression $Na_R - Na_P/D_R - D_P$ is the sodium concentration of the surplus of urine excreted during hydroflumethiazide administration.

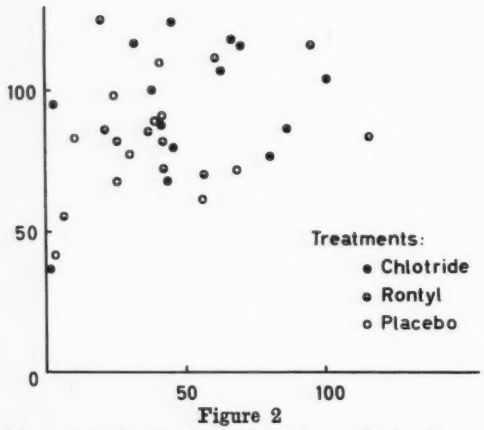

Figure 2

Magnesium (ordinate) *and calcium* (abscissa) *excretion, mg./24 hours, during permutation trial.*

From the data presented in table 2 it was shown for all 6 permutations that the sodium concentration and the chloride concentration (calculated in a similar way) of the additional urinary output during hydroflumethiazide action were far above serum levels, the mean concentrations for sodium and chloride being 353 and 407 mEq. per liter. The corresponding mean concentrations during chlorothiazide action were 176 and 122 mEq. per liter, but these concentrations are not significantly higher than the serum levels, since 2 of the 6 determinations showed values below the serum levels.

For all 6 permutations the sodium and the chloride concentrations were higher during hydroflumethiazide than during chlorothiazide action. The probability that this should be due to chance is ½ to the sixth power or less than 2 per cent.

Discussion

Variability in response to drugs is a problem in the design and interpretation of nearly all clinical experiments. It has been recognized for a long time that less variability in response will be found if the patient is used as his own control instead of treating 2 or more groups of patients with different diuretic agents.

The major problem has been, however, that the response to the drug changes during treatment, when edema is removed and the clinical condition improves. When an effective diuretic agent is given for a period of 4 or 5 days,[7] it may remove the major part of the edema the first 2 days. A diuretic agent of less potency may induce a smaller diuresis, which may, however, persist over a longer period and result in a nearly equal total loss of fluid in the course of 5 days. To avoid too large variability the test period should be so short that edema is not completely removed. By high doses of chlorothiazide or its derivatives edema may be removed so rapidly that constant body weight is attained in 3 days.[9] Statistical analysis of this study shows that the response varied from day to day. In contrast to the controlled clinical experiment in which all factors but one are controlled, the permutation arrangement of treatments permits a comparison of the effects of diuretic agents (or various dose levels of the same diuretic agent) during the whole period of dynamic changes when edema is removed. The reason for avoiding complete removal of edema even with this design is that the difference in effect between diuretics of different potency will be reduced when the available sodium stores are emptied. On the other hand, if the test period for each drug is too short, the effect of the preceding diuretic agent will still be present when the next drug is given. A confounding of effects will be produced and the difference in response between diuretics will vanish and the placebo effect increase.

It should be borne in mind that differences in acute effects may elicit different responses during chronic use. Some of these responses may be compensatory. Acute experiments do not therefore abolish the need for prolonged experiments, but the planning of such experiments is considerably simpler.

Some authors have tried to reduce the variability in response in acute experiments by equilibrating the patient on a diet containing exactly 50 mEq. of sodium daily until "a constant amount of sodium which approximates 90 per cent of their dietary sodium"[10] has been excreted. A patient in equilibrium is

Table 3

Variance of Excretions of Electrolytes and Water

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Sodium				
Treatments	2	53446.75	26723.38	30.8 (F_{99}: 8.65)
Days	2	8518.77	4259.38	4.9 (F_{95}: 4.46)
Permutations	5	44613.77	8922.75	10.3 (F_{99}: 6.63)
Error	8	6945.81	868.22	
Total	17	113525.10		
Comparisons				
$\frac{(C-R)^2}{n\Sigma\ \lambda^2}$	1	1386.75	1386.75	1.6 (F_{95}: 5.32)
$\frac{(R+C-2P)^2}{n\Sigma\ \lambda^2}$	1	52060.00	52060.00	73 (F_{99}:11.26)
Potassium				
Treatments	2	1192.33	596.16	35.9 (F_{99}: 8.65)
Days	2	112.00	56.00	3.4 (F_{95}: 4.46)
Permutations	5	687.17	137.43	8.3 (F_{99}: 6.63)
Error	8	133.00	16.625	
Total	17	2124.50		
Comparisons				
$\frac{(C-R)^2}{n\Sigma\ \lambda^2}$		200.08	200.08	12.0 (F_{99}:11.26)
$\frac{(R+C-2P)^2}{n\Sigma\ \lambda^2}$		992.25	992.25	59.7 (F_{99}:11.26)
Chloride				
Treatments	2	45097	22549	17.5 (F_{99}: 8.65)
Days	2	4291	2145	1.7 (F_{95}: 4.66)
Permutations	5	37346	7469	5.8 (F_{95}: 3.69)
Error	8	10306	1288	
Total	17	97040		
Comparisons				
$\frac{(R-C)^2}{n\Sigma\ \lambda^2}$	1	3072	3072	2.4 (F_{95}: 5.32)
$\frac{(R+C-2P)^2}{n\Sigma\ \lambda^2}$	1	42025	42025	32.6 (F_{99}:11.26)
Water				
Treatments	2	1528769	764384	38.9 (F_{99}: 8.65)
Days	2	174444	87222	4.4 (F_{95}: 4.46)
Permutations	5	3581891	716378	364.9 (F_{99}: 6.63)
Error	8	157070	19634	
Total	17	5442174		
Comparisons				
$\frac{(C-R)^2}{n\Sigma\ \lambda^2}$		527102	527102	26.8 (F_{99}:11.26)
$\frac{(R+C-2P)^2}{n\Sigma\ \lambda^2}$		1001667	1001667	51.0 (F_{99}:11.26)

$\Sigma\lambda^2$: Sum of quadrates of the sums making up the comparison.[8]

usually free of edema, and it may be questioned whether he would react similarly to patients with cardiac edema. Furthermore, the technic is time-consuming (3 weeks to test 3 doses) and necessitates many analyses of food and urine.

Since all patients in the present study had moderate cardiac edema, the results are valid only for this population. No conclusions can be drawn with regard to other populations, such as patients with hepatic or renal edema. No attempts were made to study differences

in effects on subpopulations, such as patients with rheumatic or arteriosclerotic heart disease.

Through the use of the mean output from each 2 patients receiving the treatments in the same sequence, the variance within patients is reduced and the statistical calculations are simplified. From the tables it is apparent, however, that the "error variance" is larger for sodium than for potassium excretion. The sodium output may vary considerably from day to day, even in the normal man eating a constant diet. It is therefore assumed that most of the "spontaneous" variability was due to other causes than variation in sodium intake and therefore that a strict standardization of the low-salt diet would not be necessary.

Current theories of electrolyte excretion assume that concentration of sodium in urine above serum level can only be established through a selective water reabsorption at a point distal to the level of sodium reabsorption, probably in the collecting tubules.[11] When hydroflumethiazide increases the sodium concentration far above serum level, this effect cannot be explained by reduced sodium reabsorption in the tubules, but must be due to increased water reabsorption. Chlorothiazide produces a similar effect,[12] although according to the present data it is quantitatively less pronounced.

In order to investigate whether this effect is independent of the antidiuretic hormone, 1.5 liters of water were given to 6 patients convalescent from conditions unaccompanied by edema, and urine was collected for 3 hours. The first day placebo tablets and the next day 200 mg. of hydroflumethiazide were given 2 hours before administration of water. The response varied considerably and the urinary output increased in only 3 cases after hydroflumethiazide. The sodium output, however, increased in all cases. The total increase in sodium output divided by the total increase in urine was 336 mEq. per liter, suggesting that free water reabsorption is increased also when antidiuretic hormone presumably is absent.

In terms of the countercurrent concept[11] this effect may be due to a change in permeability for water or through an increase in the osmolal gradient between the extracellular compartment in the renal papilla and the collecting tubules. Whether this effect is an acute one or whether it also is present during prolonged use of hydroflumethiazide (and accordingly of importance in the treatment of diabetes insipidus) remains to be investigated. The permutation trial is not suited for a more thorough investigation of physiologic mechanisms, but may as this example shows be useful as a screening test.

The permutation arrangement is only advantageous when the extraneous variation, such as variation among days or among patients, is significantly larger than the variation within patients. Its success also depends on whether or not the objective results of treatments can be expressed numerically. Therefore in probably rather few other fields of clinical medicine can it be applied. Its force in the investigation of the effects of diuretics is that the permutation trial can be used even in the ordinarily equipped hospital without serious interference with current treatment.

Summary

In order to increase the amount of information per day of observation the permutation trial is introduced in the study of acute effects of diuretic agents.

Chlorothiazide and hydroflumethiazide in optimal doses (2 Gm. and 200 mg. daily) and placebo tablets were given, 1 drug on each of the 3 days of the study, in all permutations to 12 patients treated by current methods for right heart failure.

The error variance of sodium, potassium, chloride, and water excretion was reduced by taking into account the variation due to permutations and days.

Chlorothiazide showed a stronger diuretic action than hydroflumethiazide, whereas the natriuretic effects were not significantly different as judged by analysis of variance. Hydroflumethiazide was less kaliuretic.

Magnesium excretion was independent of

diuresis. Neither of the diuretics showed any effect on magnesium and calcium excretion. The ratio of sodium and chloride excretion was significantly lower during hydroflumethiazide than during chlorothiazide action.

The calculated sodium concentration in the additionally excreted urine during hydroflumethiazide administration was far above serum levels (the mean of 12 observations was 353 mEq. per liter), and significantly higher than during chlorothiazide action. This effect was also demonstrated in 6 control patients during water loading, suggesting that hydroflumethiazide increases free water reabsorption also when antidiuretic hormone is absent.

Comparison of the acute effects of diuretic agents is through the permutation trial made possible in the ordinarily equipped hospital without serious interference with current treatment.

Summario in Interlingua

Pro augmentar le quantitate de information obtenite per die de observation, le essayage comparative per administrationes in ordines permutational es introducite in le studio del effectos acute de agentes diuretic.

Chlorothiazida e hydroflumethiazida in dosage optimal (2 g e 200 mg per die, respectivemente) e tablettas de placebo esseva administrate, un del medicationes per die durante le 3 dies del studio, in omne le permutationes possibile, a 12 patientes qui se trovava sub tractamento pro discompensation dexterocardiac secundo le currente methodos. Le variantias de error pro le excretion de natrium, kalium, chloruro, e aqua esseva reducite per prender in consideration le variation causate per le permutationes e le dies.

Chlorothiazida e hydroflumethiazida in dosage optimal esseva equalmente diuretic. Hydroflumethiazida monstrava un plus forte action chloruretic. Le action natriuretic del duo non differeva significativemente secundo le analyse del variantia. Hydroflumethiazida esseva minus kaliuretic.

Le excretion de magnesium non dependeva del diurese. Nulle del duo diureticos exhibiva un effecto super le excretion de magnesium e de calcium. Le proportion del excretion de natrium a illo de chloruro esseva significativemente plus basse post le administration de hydroflumethiazida.

Le calculate concentration de natrium in le surplus de urina excernite durante le administration de hydroflumethiazida esseva multo plus alte que le correspondente concentration in le sero (con un valor medie de 12 observationes de 353 mEq per litro); illo esseva significativemente plus alte durante le administration de hydroflumethiazida que durante le administration de chlorothiazida. Iste effecto esseva etiam demonstrate in 6 patients durante cargation con aqua, un facto que suggere que hydroflumethiazida augmenta le reabsorption de aqua libere mesmo quando le hormon antidiuretic es absente.

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Pulmonary Artery Wedge Catheter Position as a Site for Injection of Indicator Substance

By G. G. Rowe, M.D., C. A. Castillo, M.D., G. M. Maxwell, M.D., C. W. Crumpton, M.D., and R. J. Botham, M.D.

THE use of indicator-dilution curves during cardiac catheterization has greatly clarified the hemodynamics of various congenital cardiovascular malformations.[1, 2] As presently used, the combination of indicator-dilution curves and oximetry is usually sufficient to establish a definitive diagnosis. In some subjects with small left-to-right intracardiac shunts, however, the usual diagnostic methods are inadequate. In attempts to prove the existence of small shunts additional diagnostic procedures may be required such as the placement of 2 catheters in the right side of the heart[3] or catheterization of the left side of the heart.[4] The present study reports the injection of indicator substance with the catheter in the pulmonary wedge position as an additional diagnostic aid.

Material and Methods

In mongrel dogs, a cardiac catheter was wedged in the pulmonary artery, and, with use of the Westinghouse intensifier, sufficient quantities of Renographin or Hypaque were injected to outline graphically the course followed by a wedge injection. Some animals were killed immediately after the procedure, and the area of the lung in which the catheter had been wedged was excised for microscopic section. In a single dog a plastic tube was inserted between the right and left ventricles in a manner similar to that used by Dillon and Schreiber.[5] The tube was gripped in an adjustable clamp, and the thorax was closed with the clamp protruding so that the size of the interventricular shunt could be controlled externally. The shunt was reduced progressively until its presence could no longer be identified clearly on the curve inscribed after injection of indicator into the pulmonary artery. Then a curve was obtained with injection into the catheter wedged in the pulmonary artery to determine whether or not the shunt could still be demonstrated. The human subjects were infants, children, and adults having routine diagnostic cardiac catheterization.

The volume of the system from the point of injection of the indicator to the end of the catheter was maintained at sufficient size to contain only slightly more fluid than the quantity of indicator to be used. The catheter was wedged, the indicator was introduced cautiously into the system, and the system was closed so that the indicator remained trapped. At the proper signal a sufficient excess of saline solution was injected to wash the indicator substance through the catheter, the pulmonary capillaries, which it obstructed, and into the pulmonary vein. The arterial sampling site was the femoral artery, and the arterial specimen was withdrawn at a rate of 25 ml. per minute with a Harvard Apparatus Company constant withdrawal pump. The indicator substance was Indocyanine green (Cardio-green), and all indicator-dilution curves were made with the Waters Conley cuvette oximeter modified for use of Cardio-green.

Results

Examination of microscopic sections from the region of the dogs' lungs where the catheter had been wedged revealed that there were small areas of hemorrhage and some staining of the lung with dye (fig. 1). The area involved, however, was not large and did not appear to be severely injured, even though several injections had been made through the excised segment. In our clinical experience none of the subjects has shown signs of localized pneumonia or infarction after the "wedge injection," which would make us believe that this procedure is contraindicated.

Cinefluorography during injection of radiographic contrast substances revealed that in-

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ection into the pulmonary artery cast a relatively dense shadow in the artery but was not seen thereafter because of dilution (fig. 2). When the injection was made in the wedge position, however, the small vessels at the end of the catheter were distended sharply and the contrast substance could be seen clearly in the pulmonary vein draining this segment of the lung (fig. 3).

In the dog it was demonstrated that under the same hemodynamic conditions the recirculation of indicator that had come through the artificially created "ventricular septal defect" could be seen more readily on the indicator-dilution curve made with injection in the wedge position than on the curve made with injection into the pulmonary artery (fig. 4).

It has been possible in several clinical cases to demonstrate diagnostically abnormal curves with injection at the wedge position when the dilution curve done with injection into the pulmonary artery was equivocal. An illustrative case history may be cited. A 3-year-old boy who had grown and developed normally, was found to have a palpable systolic thrill and grade-III systolic murmur in the lower left intercostal spaces parasternally. His electrocardiogram was within normal limits, and on roentgenographic examination the heart was at the upper limits of normal in size with a questionable increase in the pulmonary vascularity. Cardiac catheterization revealed normal pressures in the right side of the heart and in the femoral artery. Oxygen saturations were 70 per cent in the mid right atrium and high right ventricle and 74 per cent in the pulmonary artery. (As calculated in volumes per 100 ml. of blood this is an increase in oxygen content from 10.6 to 11.3.) Indicator-dilution curves from the right pulmonary artery and from the right pulmonary artery wedge position are shown in figure 5. Although there may be early recirculation on the washout slope of the indicator-dilution curve done with injection into the right pulmonary artery proper, there is no doubt that this early recirculation is seen more clearly on the indicator-dilution curve made with injection into the wedge position. The indicator-dilution curves done with injection at comparable positions on the left pulmonary artery confirmed those on the right.

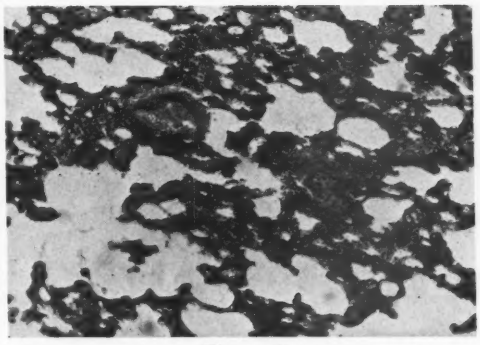

Figure 1

Photomicrograph of the portion of the lung through which a wedge injection was made. Two small areas of hemorrhage are seen. (The most damaged portion of the lung revealed by microscopic section is shown.)

There would be some doubt from the routine catheterization of this subject that a shunt existed, whereas the clinical picture was that of a small ventricular septal defect. When the "wedge injection" indicator-dilution curves (fig. 5) are considered, however, the clinical diagnosis of a small ventricular septal defect seems confirmed. Final proof of this diagnosis by cardiac surgery has not yet been accomplished, since the child's physical state appears too satisfactory to justify surgical intervention at this age.

Discussion

The hypothesis on which this study was undertaken was that, with injection into the pulmonary wedge position, the "central volume" would be reduced to the smallest size that could be obtained with a cardiac catheter passed through the right but without entering the left side of the heart. Injection at this site should produce a curve with an earlier appearance time, a sharper peak, and a narrower spread. This hypothesis was confirmed

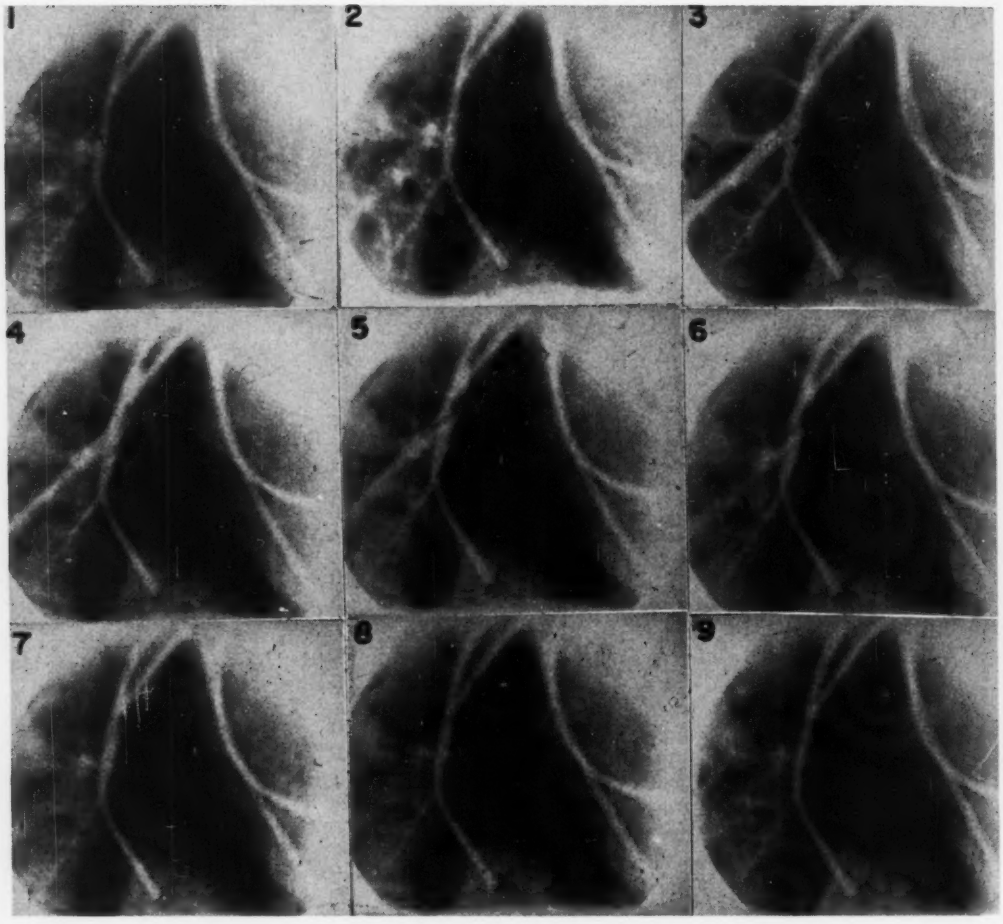

Figure 2

Consecutive pictures from a cineangiogram have been mounted to show the course of the indicator during an injection through a cardiac catheter with its tip lying free in the pulmonary artery. Although the pulmonary artery and its branches are outlined clearly, the dye is so diluted on its return through the pulmonary veins that it cannot be seen. The second cardiac catheter is in the "wedge position" and the obstructed wedge of lung contains some contrast substance that has not been washed out from previous injections.

in the dog by indicator-dilution curves and by cineangiocardiograms showing that injection through the wedge position outlines clearly the small vessels distal to the tip of the catheter and the pulmonary vein prior to its entry into the left atrium, and, if the period of injection is relatively short, the indicator substance reaches the left side of the heart in a relatively concentrated form (fig. 3). Since a high, peaked concentration of indicator traverses the left atrium and ventricle, the fraction of indicator carried through a cardiac defect by a left-to-right shunt should be relatively concentrated. Hence both the "general" and "shunt" circulation curves are sharpened, and their peaks may be iden-

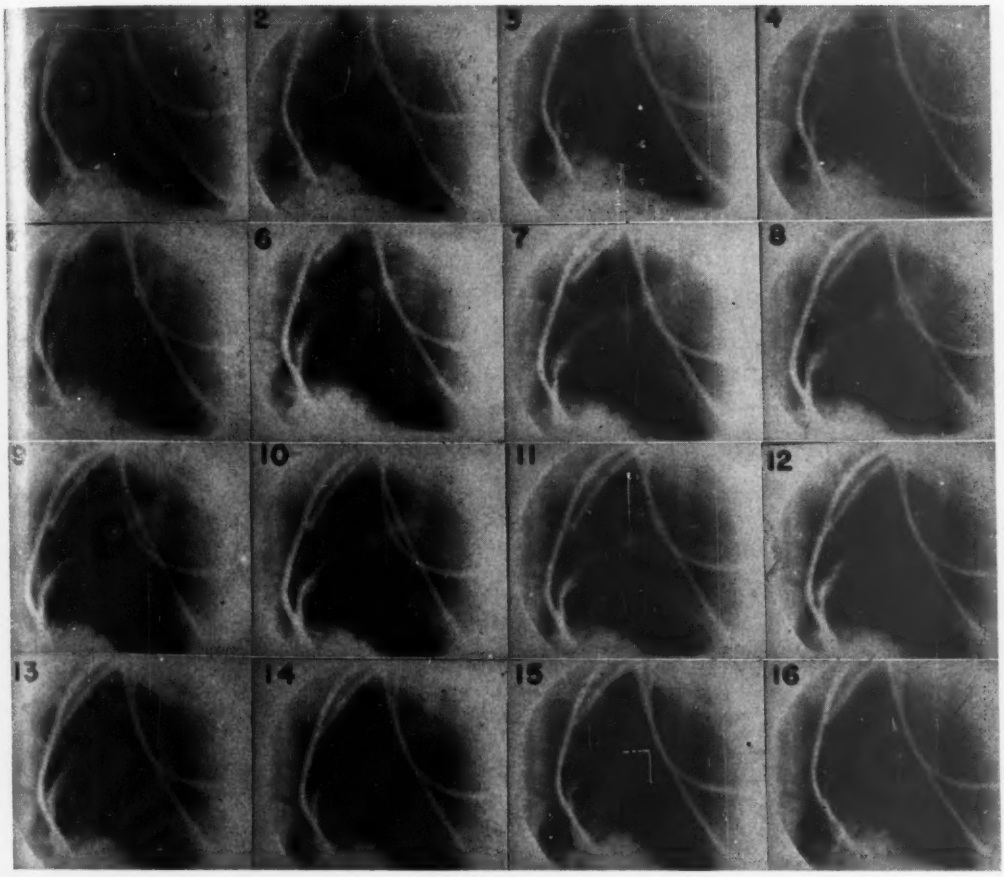

Figure 3

Consecutive pictures from a cineangiogram have been mounted to show the course of the indicator during an injection through a cardiac catheter with its tip in the wedge position. The other cardiac catheter is lying with its tip free in the pulmonary artery. The small pulmonary vessels distal to the "wedged" catheter were filled with contrast substance that extended, essentially undiluted, into the pulmonary vein draining the "wedged segment." Saline was not injected to wash out the obstructed segment of vasculature and considerable quantity of contrast substance remained in the pulmonary vessels. This is seen to clear slowly in a manner that might distort the washout slope of an indicator-dilution curve, pointing up the necessity of an adequate "flush" through the system after the injection of indicator.

tified more readily on the resulting composite curve.

In normal human subjects the "wedge injection" curves are as described for the dog and show no abnormality in their washout slope providing the indicator is washed through by sufficient volume of fluid (5 ml.) that the catheter and the section of the lung distal to it in the wedge position are well cleared of indicator. If this is not done, it appears clinically and from the cineangiocardiographic studies (fig. 3) that indicator substance may remain in the vessels obstructed by the wedged catheter and drain slowly into the circulation, distorting the washout slope of the indicator-dilution curve.

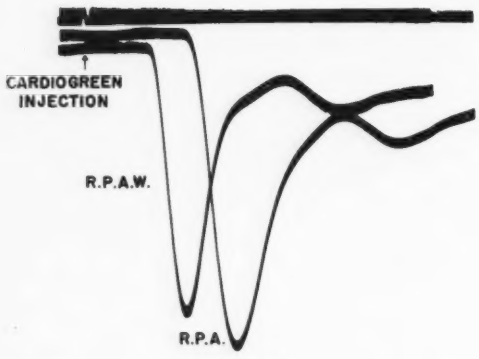

Figure 4

These indicator-dilution curves were obtained from a dog with a tube inserted between the right and left ventricle to simulate a ventricular septal defect. The amount of shunt was reduced progressively until the "RPA" curve was obtained by injecting Indocyanine green into the pulmonary artery and recording the indicator-dilution curve at the femoral artery. Immediately thereafter, without changing the shunt, the "RPAW" indicator-dilution curve was obtained after injection in the "wedge position." The RPAW curve shows the earlier appearance time, sharper peak, and narrower spread, and reveals the "shunt" recirculation more clearly, demonstrating the principle of "wedge injection." The curves are actual size and were superimposed photographically.

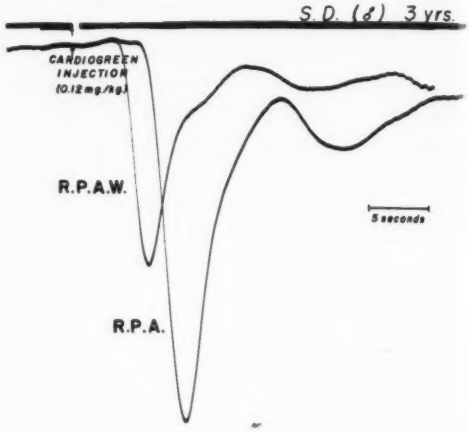

Figure 5

These indicator-dilution curves were obtained from a 3-year-old boy with a suspected ventricular septal defect. Clear-cut differences are apparent in the morphology of the curves obtained by injecting into the right pulmonary artery (RPA) and the right pulmonary artery wedge (RPAW) position. The curves are actual size and were superimposed photographically.

The passage of the indicator through the obstructed segment is dependent, not only upon the rate of blood flow through the segmental vein from collateral vessels that bypass the vessels obstructed by the wedged catheter, but also the rate of flow through this segment of the indicator and the fluid that is used to wash the indicator through the catheter. Hence, the exact portion of the "central volume," which is calculated from the curve, will depend to some extent on whether the indicator flows more rapidly through the pulmonary vein draining the segment into which the injection is made than blood flows through the other unobstructed pulmonary veins. In the absence of specific knowledge of what blood is "temporally coincident" with the injected indicator the calculation of the "central volume" could conceivably be distorted by almost as much as the volume of the pulmonary venous tree.

The significance of shunts that are too small to be shown by the standard methods of catheterization may be questioned. Very small shunts, however, may be present in subjects with marked pulmonary hypertension but with sizable defects, and it may be important to demonstrate these shunts in order to differentiate patients with septal defects from those with idiopathic pulmonary hypertension. Furthermore, some of the subjects in the present series who had the expected physical findings of a small ventricular septal defect were not shown to have any abnormality by the standard method of catheterization, including multiple indicator-dilution curves. Injection of indicator substance through the wedge position clearly indicated early recirculation on the washout slope of the curve and a central shunt. Similar, or perhaps even greater discrimination may be afforded the indicator-dilution methods by use of 2 cardiac catheters in the right side of the heart with performance of simultaneous dilution curves

from a peripheral artery and a right-sided chamber proximal to the site of injection,[3] or by left heart catheterization and injection of indicator into either the left atrium or the left ventricle,[4] but these methods are more complicated. The demonstration of such a small lesion by an objective method is intellectually more satisfying than the statement that such a lesion exists but is too small to be demonstrated.

Conclusions

The pulmonary wedge position is described as a site for injection of indicator-dilution substance because it presents the most central point in the circulation that can be attained during right heart catheterization without actually entering the left side of the heart. Injection at this site furnishes a small "central volume" and produces curves that for the same quantity of dye injected present a sharper peak concentration, a shorter build-up and disappearance time, and therefore a curve the morphology of which is more rigidly set than those obtained by injection at more proximal sites in the right side of the heart. Because the curve is sharper, small recirculation curves on the washout slope are more readily apparent; therefore smaller shunts can be detected during diagnostic cardiac catheterization.

Acknowledgment

We are happy to acknowledge the help of Dr. E. Valdivia who prepared the histologic sections of the dogs' lungs.

Summario in Interlingua

Le position del catheter inclavate in le arteria pulmonar es describite como un sito de injection del substantia indicatori proque illo representa le puncto le plus central in le circulation que pote esser attingite in catheterismo dextero-cardiac sin de facto penetrar le latere sinistre del corde. Le injection a iste sito provide un micre "volumine central" e produce curvas que—sin augmentar le quantitate de colorante injicite—exhibi un plus acute concentration culminal, un plus curte tempore initiatori e disparitori, e assi un configuration con un plus rigidemente establite morphologia in comparation con le resultatos obtenite per medio de injectiones in plus proximal sitos al latere dextere del corde. Proque le curvas es plus succincte, micre curvas de recirculation in le declivitate disparitori es plus prestemente apparente e plus micre shuntings pote esser detegite durante le catheterismo cardiac effectuate con objectivos de diagnose.

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Knowledge and Wisdom

The young man knows the rules, but the old man knows the exceptions.—Oliver Wendell Holmes, M.D. *Medical Essays*, 1861.

Changes in the Character and Location of Arterial Lesions in Mammals and Birds in the Philadelphia Zoological Garden

By H. L. RATCLIFFE, SC.D., T. G. YERASIMIDES, D.V.M., M.S., PH.D., AND G. A. ELLIOTT, D.V.M., M.S.

THE FREQUENCY of arteriosclerosis (intimal thickening with or without lipid deposits) in mammals and birds of the Philadelphia Zoological Garden has increased 10-fold since 1935.[1] This increase has involved increases in the varieties of animals that develop the disease and has been accompanied by changes in the location and character of the lesions, as well as in the apparent rate of their development. Thus, in recent years lesions of the coronary arteries have become relatively common and, since 1950, have led to deaths of a number of younger animals. In the earlier material, coronary lesions usually were limited to an occasional aged animal that had died of other causes.

The recent paper on frequency listed the varieties of mammals in which arteriosclerosis has developed most often since 1935.[1] This paper also described the conditions that have been related to the rise in frequency. The changes in the character and location of arteriosclerotic lesions in the animals of this zoo will be described and discussed at this time.

Material

The material for this report has been taken largely from autopsies of mammals and birds that have died in the Philadelphia Zoo since 1935. Earlier material from this series of autopsies has been described and illustrated.[2] It is reviewed briefly for comparison with the more recent examples of the disease.

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During 1935 the traditional and often inadequate diets then common to zoological gardens were replaced in the Philadelphia Zoo by controlled diets. Since then nutritional disease has ceased to be a factor in morbidity or mortality. These diets and the conditions under which the material from this zoo has been collected and prepared for study have been described fully.[1, 3, 4]

Observations

Prior to 1935 and the introduction of adequate diets the most striking form of arteriosclerosis developed in birds as atheromata of the thoracic aorta and brachiocephalic arteries. These lesions probably attained their largest size in the parrots and their relatives (Psittacidae), especially in parrots of the genus *Amazona*. In these birds the thickness of the atheromata often exceeded the diameters of the arteries themselves and, at times, convulsive seizures experienced by parrots could be attributed to partial obstruction of the brachiocephalic arteries by these lesions. However, this was not exclusively a disease of the psittacine birds. Relatively large atheromata also developed in the thoracic aorta and brachiocephalic arteries of ducks and geese (Anatidae), of pheasants (Phasianidae), of carnivorous birds (Accipitridae), and of the birds-of-Paradise (Paradisidae). Occasional birds of these groups also experienced convulsive seizures that could be related to the lesions.

The microscopic appearances of these atheromata were relatively uniform: loosely arranged fibrous structures with a considerable lipid content. The bases of these lesions usually extended into the media of the arteries, to involve the inner third of this coat and, occasionally, as much as its inner half.

During the same period, atherosclerosis of mammals apparently was limited to the infrahuman primates, chiefly Old World monkeys and baboons (Cercopithecidae). Occasional animals of this group developed relatively small atheromata in the abdominal aorta; these lesions were limited to the intima. In other mammals of the series the chief evidence of arterial disease was segmental necrosis and calcification of the media of the lower thoracic and abdominal aorta. These lesions corresponded more or less closely to Mönckeberg's sclerosis in man. Usually they were accompanied by thickening of the overlying intima.[2]

Within a year after improved diets had been introduced in the Philadelphia Zoo the appearance and location of the arteriosclerotic lesions, as seen during routine autopsies, seemed to be undergoing changes in both mammals and birds. Thus, in time, the large atheromata of the thoracic aorta and brachiocephalic arteries became rare in birds of all groups. Instead of these larger, softer lesions, birds now developed smaller, more compact atheromata, which have been located most often in the abdominal aorta and its branches. These more recent lesions usually have been limited entirely to the intima. That is, they differ from the atheromata that were seen before 1935 both in their structure and in the degree to which they penetrate the wall of the arteries. Their more common microscopic appearances are illustrated in figure 1 *a*, *b*, and *c*.

Since 1935 occasional examples of the Mönckeberg type of aortic disease still have been found in mammals. The increased frequency of arteriosclerosis in mammals of the Philadelphia Zoo, however, reflects the development of intimal thickening in larger or smaller foci, with or without lipid deposits, in the aorta and its larger branches, as well as in the arteries of the myocardium, the kidneys, the spleen, and other organs.

Atherosclerosis of the aorta and its larger branches rarely has contributed to the deaths of mammals or birds of this zoo. In recent years and chiefly since 1950, however, intimal thickening of smaller arteries, particularly of the intramural arteries of the heart, has become a relatively frequent cause of morbidity and mortality. Thus, a description of these lesions of the coronary arteries, and of the conditions under which they have developed in both mammals and birds, may be of considerably more interest than continued concern with atheromata of the aorta and its branches.

Arteriosclerosis of the coronary arteries and associated myocardial damage have developed in a wide variety of animals in the Philadelphia Zoo. The range in body types and food habits of these animals may be illustrated adequately by citing examples from the records of 9 families of mammals and 5 families of birds, which also are among those that have had considerable increases in the frequency of arteriosclerosis.[1] These 14 families and their records are listed separately in table 1.

This list of mammals is headed by the Bovidae, which are ruminants—buffalo, sheep, goats, and antelopes. The second family, the Macropodidae, are herbivorous marsupials—kangaroos, wallabys, and wallaroos. The Capromyidae, third, are large fur-bearing rodents, and omnivorous, according to the feeding system of this zoo.

Three families of primates follow in the list. The Cebidae are New World monkeys and include squirrel, cebus, spider, and woolly monkeys. The Cercopithecidae are Old World animals—monkeys and baboons. The Pongidae are anthropoid apes, represented here by gibbons, chimpanzees and orangutans.

Three families of carnivores complete the list of mammals. The Mustelidae include mink, skunks, otters, and badgers; the Canidae, wolves, foxes, and coyotes; and the Felidae, tigers, lions, leopards, and other cats.

Birds are represented in this series by ducks, geese, and swans—Anatidae; by pheasants and quail—Phasianidae; by parrots and macaws—Psittacidae; by cranes—Gruidae; and by hawks and related species—Accipitridae.

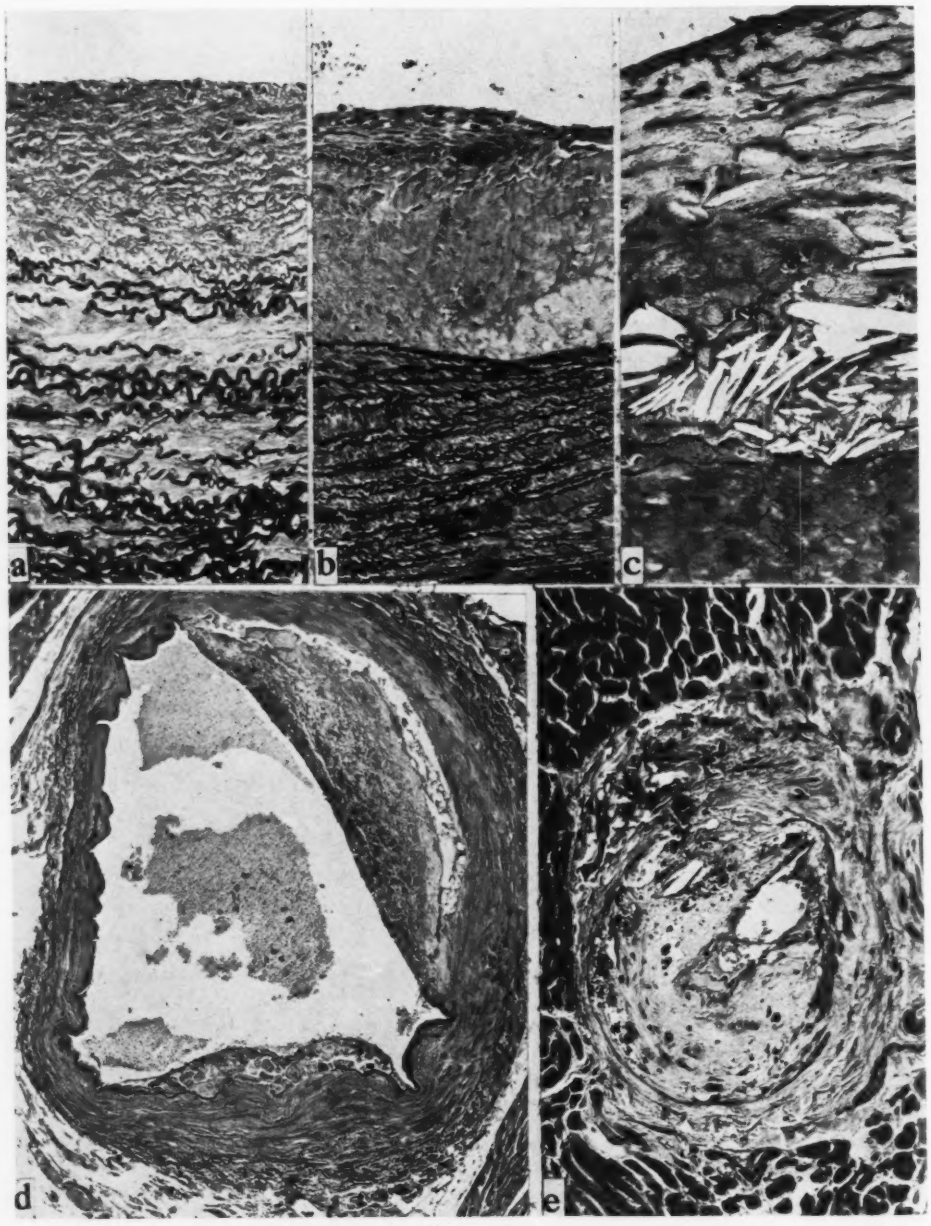

Figure 1

a *and* b, *atheromata of the thoracic aorta of geese (Anatidae)* × *200 and* × *100 respectively;* c, *atheromata of the abdominal aorta of an Impeyan pheasant (Phasianidae)* × *100;* d, *cross section through the proximal segment of the left coronary artery of a European badger (Mustelidae)* × *50;* e, *cross section through a proximal branch of the left coronary at a focus of thrombosis and recanalization: douroucouli (Cebidae)* × *100. (*a *through* e, *Verhoeff's elastic tissue stain.)*

Table 1

Families of Mammals and of Birds in Which Intimal Disease of the Coronary Arteries Has Been Associated with Fibrosis and Infarction of the Myocardium Since 1945

Families:	HEART						Sex	
	Arteries		Myocardium					
	deep	surface	fibrosis	infarcts	Aorta	Spleen	♂	♀
Mammals								
Bovidae	13	3	10	3	6	9	3	10
Macropodidae	10	1	4	3	4	6	2	8
Capromyidae	10	1	6	4	3	3	6	4
Cebidae	9	1	4	3	1	5	4	5
Cercopithecidae	9	1	2	0	3	7	2	7
Pongidae	7	0	4	2	1	1	5	2
Mustelidae	9	2	6	2	2	4	4	5
Canidae	17	2	7	3	4	11	10	7
Felidae	12	0	6	2	2	3	7	5
Totals	96	11	49	22	26	49	43	53
Birds								
Anatidae	28	3	15	9	13	13	15	13
Phasianidae	13	1	4	2	8	10	6	7
Psittacidae	20	3	8	5	12	11	9	11
Gruidae	12	0	5	4	9	9	4	8
Accipitridae	11	2	5	4	6	6	8	3
Totals	84	9	37	24	48	49	42	42

Animals listed for each family were selected as examples of coronary disease. In about half of them arteriosclerosis was associated with damage to the myocardium. The relative frequency of aortic and splenic arteriosclerosis also is shown.

Birds of the last-named group are carnivores. All the others have received a diet designed for omnivorous animals.

Table 1 presents a summary of observations of 96 mammals and 84 birds of the 14 groups. These examples of coronary disease were collected between January 1, 1945, and December 31, 1958. Each animal of this series was found to have developed intimal thickenings of the coronary arteries that at least equaled the thickness of the medial coat of the vessels. Lesions of this dimension were the requirement for inclusion in the table.

The first 2 columns of the table show the relative frequency with which the deep and the superficial arteries of the myocardium were involved. Disease of the surface arteries always was accompanied by disease of the intramural vessels. The reverse obviously has not been so.

The columns under "myocardium" show the frequency of fibrosis and of infarction in this series. A majority of examples of infarction were combined with fibrosis of the myocardium.

It also seemed of interest to show in this table the relative frequency of atherosclerosis of the aorta, and of intimal thickening of the arteries of the spleen in this series of mammals and birds. The last 2 columns list the animals by sex.

About 10 per cent of the mammals and birds of this series had developed atheromata of the larger coronary arteries. These lesions, whether they developed in mammals or in birds, corresponded closely in both macroscopic and microscopic appearances to lesions of the same sites in man (fig. 1*d* and 1*e*). The occurrence of this type of coronary lesion does not appear to have been related to the size of the animal or to the type of diet. Atheromata have been found in the larger coronary arteries of animals that ranged from large bovines to small monkeys and carnivores, and in representatives of 4 of the 5 families of birds. The ages of animals in which these lesions developed also ranged widely, but in a majority of the cases they appeared in the older animals. Sex apparently was not directly related to their development.

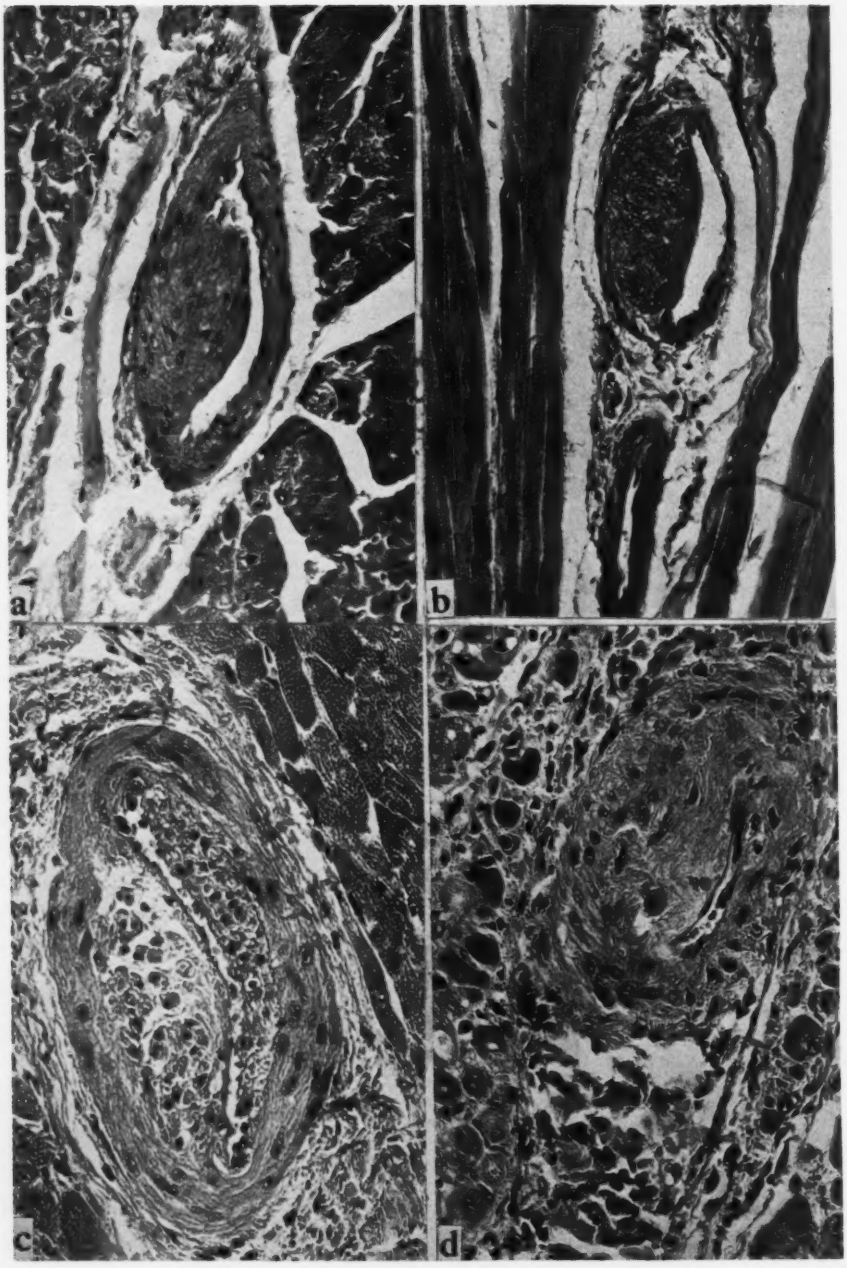

Figure 2

Intimal thickening of the intramural branches of the coronary arteries in the walls of the left ventricle: a, *from a crowned crane (Gruidae);* b, *from a kalege pheasant (Phasianidae);* c, *from a timber wolf (Canidae); and* d, *from a snow leopard (Felidae). The lesions seen in* c *and* d *were associated with infarction; necrotic muscle lies adjacent to the occluded artery in* d. *All* × *200. (*a *and* b. *Verhoeff's stain,* c *and* d, *hematoxylin and eosin.)*

In this series of animals the atheromata of the coronaries have been accompanied by more or less intimal thickening of the smaller intramural branches of the arteries, and also in some instances by scars in the myocardium. The values in table 1, however, show that neither fibrosis nor infarction was dependent upon the occurrence of atheromata in the larger arteries. In fact, none of the examples of infarction in this series was associated with atheromata in the larger arteries. Instead, intimal disease of the deeper intramural arteries apparently has been the only cause of myocardial infarction or fibrosis.

Figures 2 and 3*a* and 3*b* illustrate some of the variations in the microscopic appearances of the lesions of the smaller vessels, which incidentally were essentially the same whether in mammals or in birds. Usually they seemed to have developed as relatively localized changes, often involving short segments of the arteries, and ranging from densely cellular to almost completely acellular homogeneous lesions.

Differential stains suggested that the more cellular of these lesions were composed of fibrous tissue. Less cellular ones contained finely divided elastic fibers or fibers that corresponded, in their staining qualities, to elastic fibers. The more homogeneous lesions lacked fibers and nuclei and perhaps are best described as "hyalinized," although occasional ones apparently contained lipid droplets.

The development of these lesions of the deeper coronary arteries accounts for both fibrosis and infarction of the myocardium. Sections of the hearts of approximately half of the mammals and birds that have been included in the table contain one or more foci of fibrosis.

Infarcts have been found in about 25 per cent of the series. Invariably, infarcts in the hearts of these animals have been multiple small lesions from 1 to 10 mm. in their greatest diameters and, with few exceptions, visible macroscopically only in the walls of the left ventricle.

Coronary disease in this series was accompanied by atherosclerosis of the aorta in about 25 per cent of the mammals and in about 50 per cent of the birds. Intimal disease of the splenic arteries was found in about half of both mammals and birds. These records are given simply to illustrate again that arteriosclerosis of the coronary arteries develops in a wide variety of animals independently of the disease in other sites.

Discussion

Improved diets for mammals and birds in the Philadelphia Zoological Garden have been associated with changes in the character and location of arteriosclerotic lesions. The first changes were noted within 5 years after new diets were introduced. Thus, they may have been related to improvement in nutrition.

More recently, beginning at least 10 years after the improved diets started, lesions of the coronary arteries have become increasingly frequent and severe. This later change has been associated with increasing population densities in the zoo, and increased breeding activity.[1, 4] Present evidence suggests that the increase has been a response to psychologic disturbances in the exhibition groups.[5–7]

The validity of this hypothesis must depend upon the results of experimental studies that are now under way. Meanwhile, it may be noted that intimal thickening and occlusions of the distal segments of the coronary arteries have been known for many years as a common disease of man.[8–10] Moreover, narrowing and occlusion at this level of the coronary system are well recognized causes of myocardial damage in man.[8, 11–13] Recently the lesion also has been described in dogs, cats, baboons, and in at least 1 "ape"—apparently a chimpanzee.[14–18] The present material demonstrates that the lesion also may develop in a great variety of mammals and birds, and in all of them may lead to infarction and fibrosis of the myocardium.

Current discussions of coronary disease of man, however, generally have been concerned with atherosclerosis of the proximal segments of the arteries.[18–20] Intimal thickening and occlusion of the smaller, intramural branches of these arteries often seem to be considered

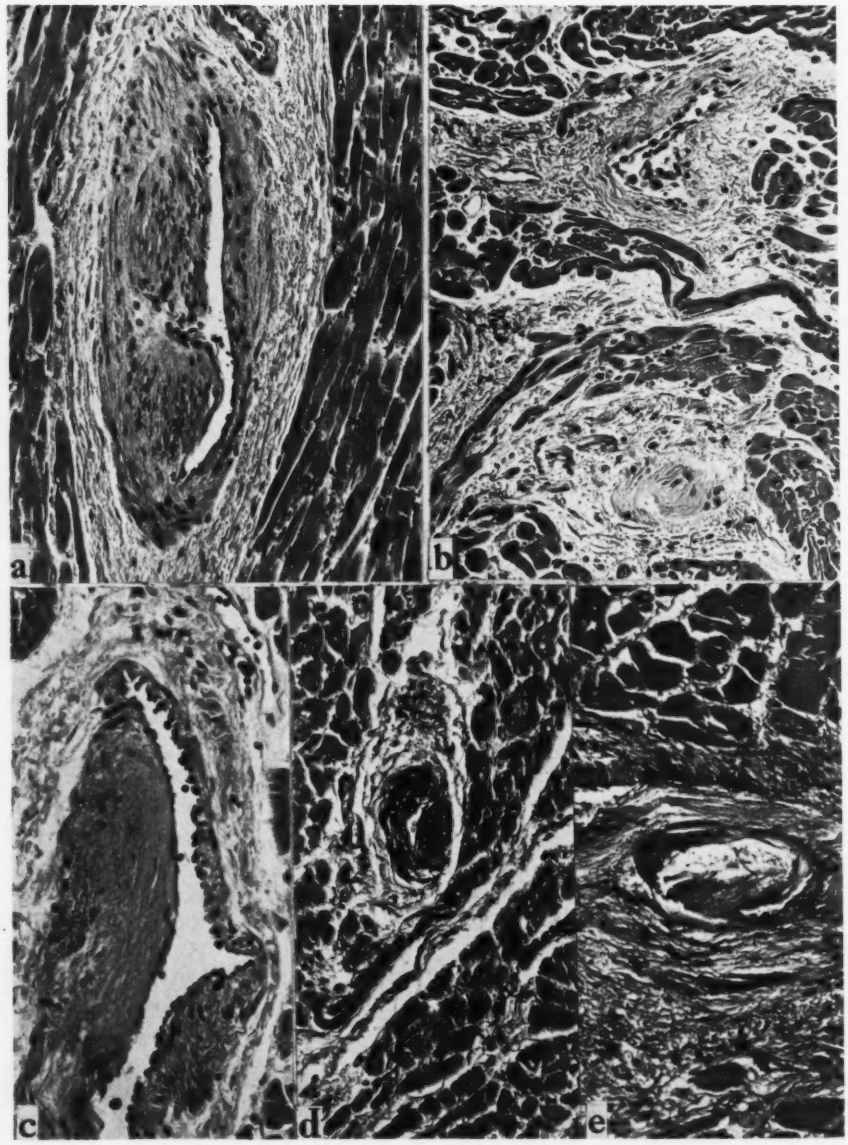

Figure 3

Intimal thickening in the intramural branches of the coronary arteries in the walls of the left ventricle: a *and* b, *male orang-utan (Pongidae) young adult, 18 years of age, captive-born in the Philadelphia Zoo. Note in* a *the perivascular fibrosis and occlusion of a lateral branch of the artery. In* b *atrophy and fibrosis of the myocardium are associated with occlusion of 2 smaller arteries;* c, d, *and* e *from man, postoperative deaths;* c *and* e *men. 70 and 68.* d *women 67 years of age. All* × *100. (*a *through* e. *Verhoeff's stain.)*

a separate entity, or else are ignored as being of little or no significance.

At this time, one opinion attributes this lesion of the smaller vessels to inflammatory processes, either of the myocardium, as in rheumatic fever, or of more generalized disturbances, as in sensitivity reactions and infections.[9, 21, 22] Others have suggested, however, that this lesion is an integral part of the whole process of coronary arteriosclerosis or atherosclerosis.[23, 24]

According to this second view, intimal thickening may be initiated in foci at any and all levels of the coronary arteries. The mechanism by which this is accomplished is unknown, but it may involve fibrosis, elastosis, endotheliosis, thrombosis, etc.[20, 23-25] The proximal segments of these vessels, by virtue of both size and anastomoses,[11] provide space and time needed for the development of the more spectacular lesion—atheroma with hemorrhage and thrombosis. More distal branches of these arteries undergo occlusion and disappear into the scar tissue that replaces the myocardium, before more conspicuous changes may take place.

Observations on lesions of the coronary arteries of both mammals and birds in the Philadelphia Zoological Garden support this latter opinion. Moreover, concurrent observations on coronary arteries of man and a series of domesticated animals are in keeping with this view (fig. 3*c*, *d*, and *e*). Certainly we have found nothing to suggest that coronary disease in these zoo animals can be related to infectious disease or to sensitization. If these conditions (infections and sensitivity) had contributed to arterial disease in these populations some of the earlier stages should have been seen, for the animals listed here are a small fraction of the number autopsied since 1945. Still, neither thrombosis nor inflammatory reactions in or about arteries have been found. Furthermore, coronary disease was unknown in these animals for about a decade after improved nutrition had virtually eliminated infectious disease as a cause of morbidity and mortality. This interval suggests, as well, that lesions of the coronary arteries have not been related to diets. Nevertheless, improved diets apparently have contributed to the development of these lesions through increased vigor and a more intense response of the animals to population densities.[1, 4]

Summary

Improved nutrition for mammals and birds at the Philadelphia Zoological Garden has been followed by continued increases in the frequency of arteriosclerosis and by changes in the character and location of the lesions. During the first decade after diets were improved the large atheromata of the proximal aorta and brachiocephalic arteries of birds were replaced by smaller, more compact lesions, usually of the abdominal aorta. At the same time many species of mammals developed atheromata of the aorta, whereas earlier these lesions had been found chiefly in baboons and monkeys (Cercopithecidae).

During the second decade of adequate nutrition, and especially since 1950, arteriosclerosis of the coronary arteries has become relatively common in both mammals and birds. Usually this lesion has developed in the distal, intramural segments of the coronary arteries, as intimal thickening and occlusion. Most frequently it has been associated with myocardial fibrosis, but it also has led to myocardial infarction and sudden death in both mammals and birds. Present evidence suggests that this lesion reflects a response of adequately nourished animals to population densities.

Summario in Interlingua

Le meliorate alimentation del mammales e aves al jardin zoologic de Philadelphia ha essite sequite per un continue augmento del frequentia de arteriosclerosis e per alterationes in le character e in le sito del lesiones. Durante le prime decennio post le melioration del dietas, le grande atheromas del aorta proximal e del arterias brachiocephalic in le aves esseva reimplaciate per plus miere e plus compacte lesiones, usualmente in le aorta abdominal. Simultaneemente multe species de mammales disveloppava atheromas del aorta, durante que previemente iste lesiones habeva occurrite principalmente in babuinos e simias longicaudate (Cercopithecidas).

In le curso del secunde decennio de adequate dietas, e specialmente depost 1950, arteriosclerosis del arterias coronari ha devenite relativemente commun tanto in mammales como etiam in aves. Usualmente iste lesion se ha disveloppate in le segmentos intramural distal del arterias coronari, in le forma de spissification o occlusion intimal. Le plus frequentemente illo esseva associate con fibrosis myocardial, sed illo ha etiam resultate in infarcimento myocardial con subite mortes in mammales e etiam in aves. Le factos que es currentemente cognoscite suggere que iste lesion reflecte un responsa de adequatemente nutrite animales al densitate del population.

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SYMPOSIUM ON REGULATION OF THE CARDIOVASCULAR SYSTEM IN HEALTH AND DISEASE

Guest Editor: LYSLE H. PETERSON, M.D.

Introduction

By LYSLE H. PETERSON, M.D.

IT IS NOW more than 300 years since Harvey described the circulation of the blood. Three hundred years is not a long time, but it includes, according to historians, more than 90 per cent of the recorded scientific achievements of man. During these 300 years enormous effort has been expended in attempting to understand the properties and behavior of the cardiovascular system. It is perhaps significant in considering this effort, which was participated in by some of the world's most outstanding scientists, that we do not have a clue to the etiology of the major diseases of the cardiovascular system. Indeed, we cannot elaborate a reliable physiologic explanation for the cardiovascular response to any of man's stresses, such as exercise for example.

In sharp contrast to this lack of a satisfactory concept of how the system works *as a system,* much is known about the properties and behavior of a myriad of the pieces and parts that comprise the cardiovascular system (fig. 1).

A great stride in understanding the cardiovascular system would be made if we could put the pieces together instead of becoming lost among them. Putting pieces together implies that there is some logical arrangement into which they naturally fit. One way in which the behavior and properties of the cardiovascular system can be organized into a logical pattern is with regard to its regulation. To do this we must first define what we mean by regulation. We have chosen to adopt the definition of regulation used by most engineers, physical scientists, and many biologists. I am told that this rigorous definition is also applied by many sociologists and economists.

The principles of a regulated system can be illustrated by considering the regulation of the temperature of a house. Figure 2 is a schematic drawing representing the elements of a system that has been designed to regulate the temperature of a house. On the left wall of the house is a thermo stat, which consists of 3 basic parts: a device to measure the actual temperature of the interior of the house (thermometer), a device that allows the occupant of the house to "set" the desired temperature, and a device that will generate some kind of signal (e.g., an electric voltage) that is proportional to the difference between the "set" temperature and the real temperature. This signal is called the "error," i.e., the error between the real temperature and the set temperature. The regulatory system is designed toward minimizing the error between the ideal or set temperature and the actual temperature, i.e., to regulate the temperature around the ideal value. The error signal from the

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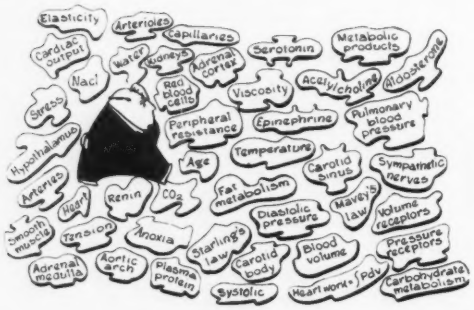

Figure 1

See text for a possible relationship to the symposium.

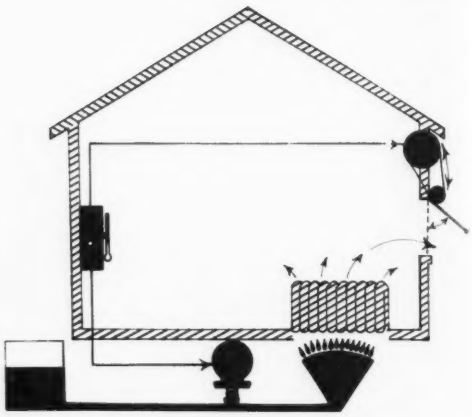

Figure 2

Schema of a temperature-regulating system. Below the floor of the house is a tank containing fuel, a motor that turns a valve that admits fuel to the furnace. Above the window on the right is a motor that controls the window. The 2 motors are connected by wires (indicated by black lines and arrows) to the thermostat on the left wall of the house. See text for a description of the function of this system.

thermostat then commands or controls the heat-producing device (furnace) and the heat-dissipating device (window). In this case, heat production is governed by controlling the motor that turns the valve, hence determining the fuel supply to the furnace. Heat dissipation is controlled by the motor that turns the gears, thus opening or closing the window. Thus, if the temperature is too low, i.e., lower than the "set" temperature, the thermostat generates an error signal that increases heat production and decreases the heat loss, hence raising the temperature of the house. Conversely, if the temperature is too high, an error signal commands the furnace and window mechanisms in such a way that the temperature will fall until it reaches the ideal level. Thus, it can be seen that the error may be either negative or positive and that there are mechanisms that can drive the temperature up or down.

There are certain important characteristics that must be present in such a system in order to establish that a function is regulated by the system:

1. We must identify the character of the function that is being regulated. In this case, it is the temperature of the interior of the house at the site of the thermometer.

2. The system must possess a mechanism for measuring the function that is being regulated, i.e., a sensor or receptor is required. In this case, it is a thermometer for measuring temperature.

3. The system must possess a device for setting the desired value of the regulated function. This value is called the ideal or "set" value. In this case, it is the temperature level selected by the occupant of the house and it is put into the thermostat by the setting of a dial.

4. The system must contain a device for comparing the real and ideal value and for generating a coded signal that is proportional to the difference.

5. The system must contain devices for changing the magnitude of the controlled function. In this case, for changing the temperature of the house, i.e., furnace and window.

6. In such a system all these functions are connected in the form of a closed loop or circuit. The furnace and window mechanisms are connected to the thermostat by wires and the thermostat is connected to the furnace and windows by the temperature of the air in the room.

7. The system should behave so that if the

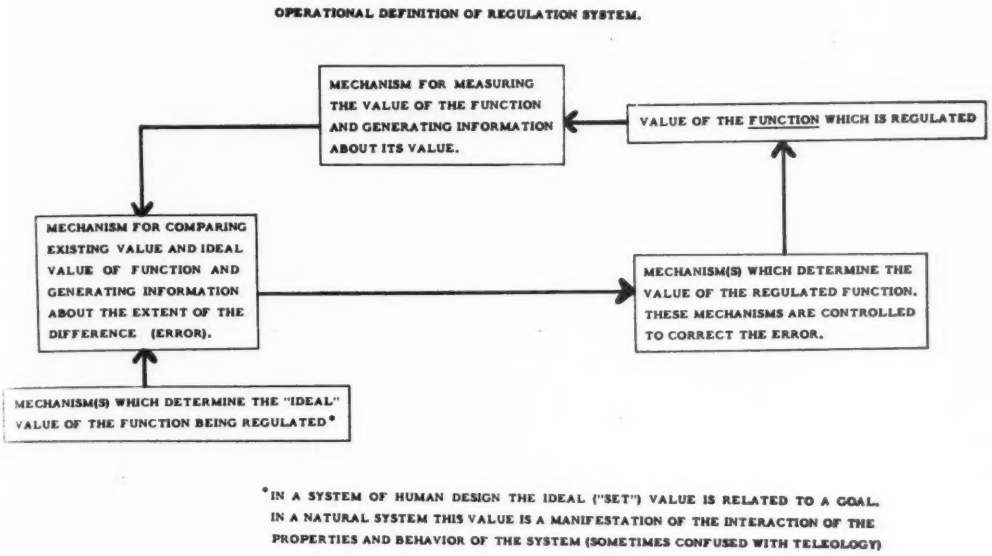
OPERATIONAL DEFINITION OF REGULATION SYSTEM.
MECHANISM FOR MEASURING THE VALUE OF THE FUNCTION AND GENERATING INFORMATION ABOUT ITS VALUE.
VALUE OF THE FUNCTION WHICH IS REGULATED
MECHANISM FOR COMPARING EXISTING VALUE AND IDEAL VALUE OF FUNCTION AND GENERATING INFORMATION ABOUT THE EXTENT OF THE DIFFERENCE (ERROR).
MECHANISM(S) WHICH DETERMINE THE VALUE OF THE REGULATED FUNCTION. THESE MECHANISMS ARE CONTROLLED TO CORRECT THE ERROR.
MECHANISM(S) WHICH DETERMINE THE "IDEAL" VALUE OF THE FUNCTION BEING REGULATED*
*IN A SYSTEM OF HUMAN DESIGN THE IDEAL ("SET") VALUE IS RELATED TO A GOAL. IN A NATURAL SYSTEM THIS VALUE IS A MANIFESTATION OF THE INTERACTION OF THE PROPERTIES AND BEHAVIOR OF THE SYSTEM (SOMETIMES CONFUSED WITH TELEOLOGY)

Figure 3

Schematic drawing of the general properties of a regulation system in order to emphasize its closed loop or circuit aspect. For description, see text.

magnitude of the regulated function is displaced from the ideal or set value, the system will respond so as to return the magnitude toward its ideal or set value. Furthermore, if the closed loop is opened or cut, the regulation will not persist.

It should be noted that the function of the circuit or closed loop arrangement of the system is to "feed" the value of the regulated variable "back" into the system in such a direction that the value of the variable (e.g., temperature) is controlled in such a way as to minimize the error. In other words, the variable itself is used to furnish the information by which control of itself is achieved and therefore it can be regulated. This form of feeding back is called "negative feedback." If the direction of the feedback is such that the magnitude of the error is increased, e.g., by increasing the output of the furnace when the temperature rises, the process is called positive feedback.

Figure 3 is a schema that identifies the aspects of a regulatory system and indicates the form of the circuit or closed loop. Most regulatory systems perform their function by means of negative feedback. This applies to biologic systems as well as man-made systems.

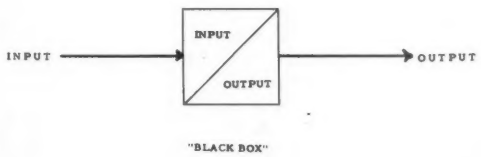

Figure 4

Essentials of a "black box." Only the relationships between the input *and* output *are considered. This relationship is called the transfer function. The mechanisms by which the output and input are related (transfer function) are the mechanism implied in the black box and are indicated by the diagonal line. For example, if* x = *the input, and if* y = *the output, only* y = 5x *then the black box contains a mechanism for multiplying the input by 5 to get the output.*

I should like to emphasize the difference in meaning between regulation and control: temperature is regulated, but the mechanisms (such as the furnace and window) for determining temperature are controlled. Another phenomenon that might be confused with reg-

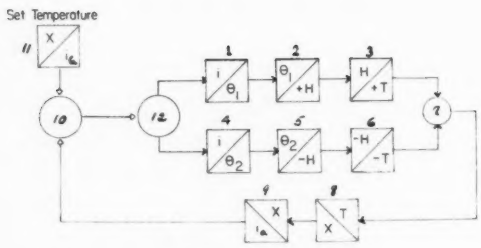

Figure 5

Schematic drawing of a temperature regulatory system for a house. The function of each element or black box according to its number is:

	Input	Output
1.	*Error signal (i)*	*Rotation of fuel valve motor (θ_1)*
2.	*Rotation of fuel valve motor (θ_1)*	*Heat production by furnace (+H)*
3.	*Heat (H) to air*	*Temperature rise (+T)*
4.	*Error signal (i)*	*Rotation of window motor (θ_2)*
5.	*Rotation of window motor (θ_2)*	*Heat loss from window (—H)*
6.	*Heat loss (—H)*	*Temperature drop (—T)*
7.	*Temperature of room (T) determined by the relative amount of heat loss and heat production*	*Temperature of room (T) determined by the relative amount of heat loss and heat production*
8.	*Temperature of room (T)*	*Movement of mercury in thermometer (X)*
9.	*Movement of mercury (X)*	*Signal that indicates mercury movement with respect to temperature (i_a)*
10.	*Coded information indicating temperature of the room is compared to coded information from the ideal temperature setting box*	*Coded information indicating temperature of the room is compared to coded information from the ideal temperature setting box*
11.	*Setting of thermostat dial (X)*	*Coded information about setting (i_b)*
12.	*Distribution of error signal (i) to furnace and window motor*	*Distribution of error signal (i) to furnace and window motor*

ulation is what might be called *"incidental control,"* e.g., it is widely held that the carbon dioxide tension of the arterial blood is *regulated* and only incidentally, i.e., because of the nature of air at sea level, arterial oxygen tension tends to remain quite constant. Thus, the arterial partial pressure of carbon dioxide is regulated while that of oxygen is incidentally controlled.

Drawing pictures such as the house in figure 2 is not always the most convenient way of representing a system in which there is interaction of many variables. There are a variety of ways, moreover, of schematizing such systems with symbols connected by arrows, etc. I should like to introduce a scheme to this symposium that is used by many scientists and engineers. The scheme involves the use of what are called "black boxes." A black box is an imaginary structure that carries out some function without indicating how the function is performed. Thus, only what goes into and what comes out of the box are considered. The advantage of the concept of the black box is that it identifies the function being considered in terms of what it puts out with respect to what it takes in, rather than how it does it. Figure 4 represents such a black box.

The black-box concept also suggests a very common technic of scientific thinking. Most

Often a scientist begins his investigation of a system by considering what it does, i.e., its input and output, rather than by how it does it. Scientists call the functional relationship between input and output the *transfer function*. They may also develop their own input or stimulus, and then measure the system's output or response to that stimulus. The black box can represent any scale of nature, e.g., from an atom to the universe. It may, for example, represent the furnace in which the input is fuel and the output is heat, or it may represent the fuel valve in which the input is an error signal from the thermostat and the output is the degree of opening of the valve. Figure 5 is an example of how the regulation of temperature of the house may be represented using black boxes. Any number of black boxes can be put into the scheme, depending upon the number of elements one wishes to consider. The closed-loop arrangement, which is one of our requirements of a regulation system, is apparent.

Many functions of the cardiovascular system have been said to be regulated. Some of these are indicated in figure 6. It is the purpose of this symposium to examine certain of these concepts in the light of today's knowledge, 300 years after Harvey, and to see how the pieces fit together—which pieces are missing and to consider whether, indeed, these functions are regulated. The considerations are all germane to our basic understanding of normal and abnormal cardiovascular functions.

I have been asked to act as a moderator; I am not sure how a moderator fits into the schema of regulation. An analogy that troubles me is that the nerve bundles coming from the aortic arch and carotid sinus regions have been called moderator nerves, but they have also been called depressor nerves; to avoid the latter function, I shall merely serve to introduce the subject of this symposium and complete my role as moderator by outlining the schedule of the symposium.

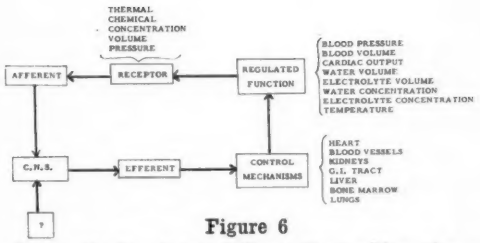

Figure 6

Schematic drawing to indicate the position of various aspects of the cardiovascular system, which forms the subject of this symposium, within the definition of regulation.

Summario in Interlingua

In su remarcas de introduction al presente symposio super le regulation del systema cardiovascular, le autor lista le characteristicas que debe esser presente in un systema particular ante que on pote asserer que un function particular es regulate per ille systema.

1. Nos debe identificar le natura del function que se trova sub regulation.
2. Le systema debe posseder un mechanismo pro le mesuration del function que se trova sub regulation, i.e., un sensor o receptor es requirite.
3. Le systema debe posseder un dispositivo pro fixar le desirate valor del function que se trova sub regulation. Iste valor es le "valor ideal."
4. Le systema debe continer un dispositivo pro comparar le valor real e le valor ideal e pro generar un signal cifrate que es proportional al differentia inter le duo.
5. Le systema debe continer dispositivos pro alterar le magnitude del function sub regulation.
6. In un tal systema, omne le mentionate functiones es connectite in le forma de un circuito claudite.
7. Le systema debe comportar se de maniera que quando le magnitude del function sub regulation es displaciate ab le valor ideal, su responsa resulta in le retorno de ille magnitude a su valor ideal. In plus, si le circuito es aperite o disrumpite, le regulation non pote persister.

In iste senso, multe functiones del systema cardiovascular ha essite considerate como regulate. Un lista de tales es presentate per le autor, incluse illos que forma le themas individual del presente symposio.

Regulation of the Heart's Functions

By ROBERT F. RUSHMER, M.D.

THE SIGNIFICANCE of cardiovascular regulation extends beyond considerations of normal function and control. Primary derangements of various cardiovascular control mechanisms represent an important and perplexing group of disease entities confronting the physician in his daily practice. The various types of regulating mechanisms being discussed in this symposium constitute a rational classification of those clinical problems that are produced by distortion or abnormalities of control. For example, abnormal control of heart rate is involved in the extravagant tachycardia that occurs with neurocirculatory asthenia and other manifestations of psychologic instability. Both primary and secondary systemic arterial hypertension involves abnormalities in blood pressure control. Orthostatic hypotension represents a failure of the normal peripheral vascular response to arising. A more specific defect in fine control of peripheral vasculature occurs in Raynaud's disease. The regulatory mechanisms that influence erythropoiesis may produce anemia at one extreme and polycythemia at the other. The control of blood volume and of total body fluids must be deranged when patients with advanced cardiac disease develop peripheral vascular congestion and edema.

In addition to primary disturbances of control mechanisms, physicians must be alert to changes in control systems induced by various organic disease states. Consider a patient with severe aortic insufficiency. In spite of voluminous uncontrollable regurgitation of blood during each cardiac cycle, the net forward flow of blood through the arterial system is generally within the range of normal values so long as the heart remains compensated. Similarly, the cardiac output tends to be maintained even after large portions of the ventricular myocardium have been replaced by scar tissue after severe coronary occlusion. On the other hand, hyperthyroidism or emotional states may impose sustained loads on the hearts of patients that are unrelated to disease of the cardiovascular system. From this point of view it becomes imperative that the basic control mechanisms of the cardiovascular system be as fully understood as possible.

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Cardiac Control

If we consider control of the heart in terms of the definition and example proposed by Dr. Peterson, many essential pieces of information are missing. Since the heart is a pump, its control must take the form of adjustments in the heart rate or stroke volume or both. If the cardiac output is regulated by a sensing element that provides an error signal whenever the cardiac output varies from some set value, we cannot at present identify the flow-sensing mechanism; we do not know the mechanism by which the resting levels are "set" nor can we describe in any detail the afferent pathways or coordinating centers for the appropriate reactions that maintain resting cardiac output. It is tempting to assume that the normal resting cardiac output represents the level to which heart rate and stroke volume would tend if all controls were removed. This concept does not appear applicable to the regulation of resting heart rate, which is the resultant of the mutually antagonistic effects of sympathetic and parasympathetic nerves to the pacemaker region. The parasympathetic effects are generally regarded as dominant, so that section of both sets of nerves leads to a faster heart rate than the normal resting value. Moreover, the cardiac index at rest

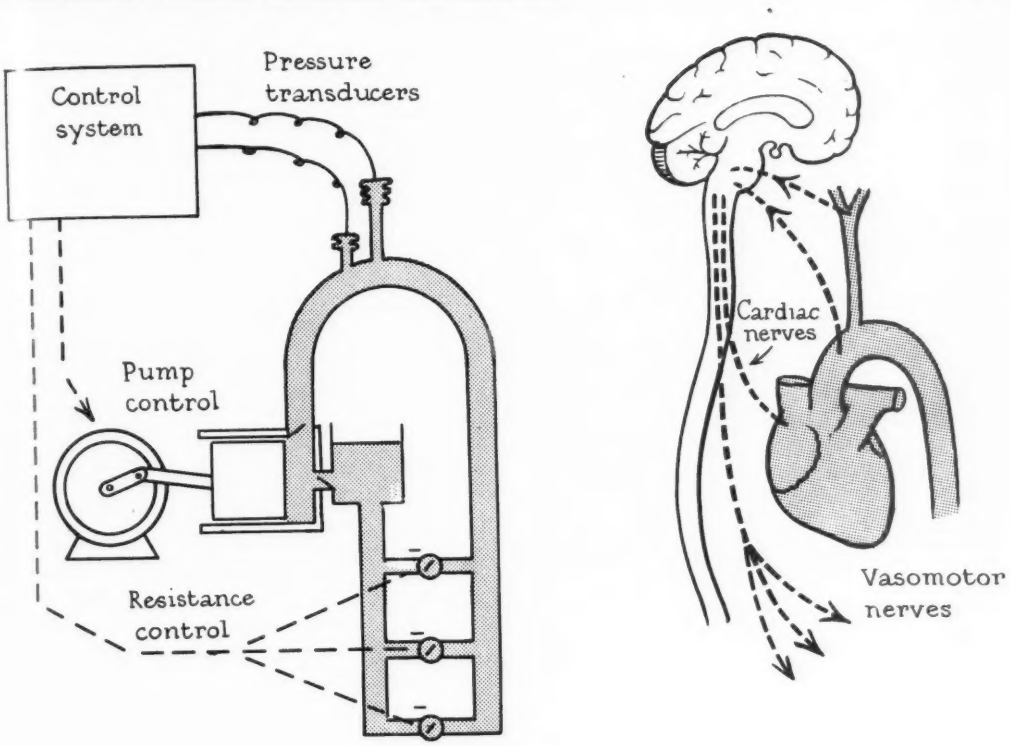

Figure 1

In a simple hydraulic system, the perfusion pressure can be maintained within narrow limits by a servo-control system that produces appropriate changes in pump output to balance any net changes in peripheral resistance or vice versa. The correspondence between this basic pattern and the pressoreceptor mechanism is illustrated.

tends to be about the same for subjects with either rapid or slow heart rates. In other words, a relatively slow heart rate is associated with a relatively large stroke volume and vice versa. If this is the case, the cardiac output may be regarded as "set" in terms of some other criteria, i.e., peripheral tissue requirements.

The flow of blood through peripheral tissues might be regulated to maintain a constant environment for the various cells. Such regulation might involve chemoreceptors in the systemic veins to monitor oxygen content of venous blood to provide sensing elements for proper adjustment of peripheral flow distribution and cardiac output. Such adjustments would be truly applicable only to those tissues in which blood flow is dependent upon metabolic rate and oxygen consumption, i.e., skeletal muscle. No chemoreceptors have been demonstrated in the various systemic veins thus far. Since skin serves an essential function in heat exchange, proper sensing elements to "set" blood flow through the subcutaneous tissues would be some form of temperature receptors. What would be the most appropriate sensory elements to monitor renal blood flow, gastrointestinal perfusion or circulation through glands? We have not found such receptors as might be required for these purposes. Indeed, in many instances we would not even know what to look for.

The traditional view of cardiac output regulation revolves about a mechanism for maintaining systemic arterial pressure to provide an effective balance between peripheral blood

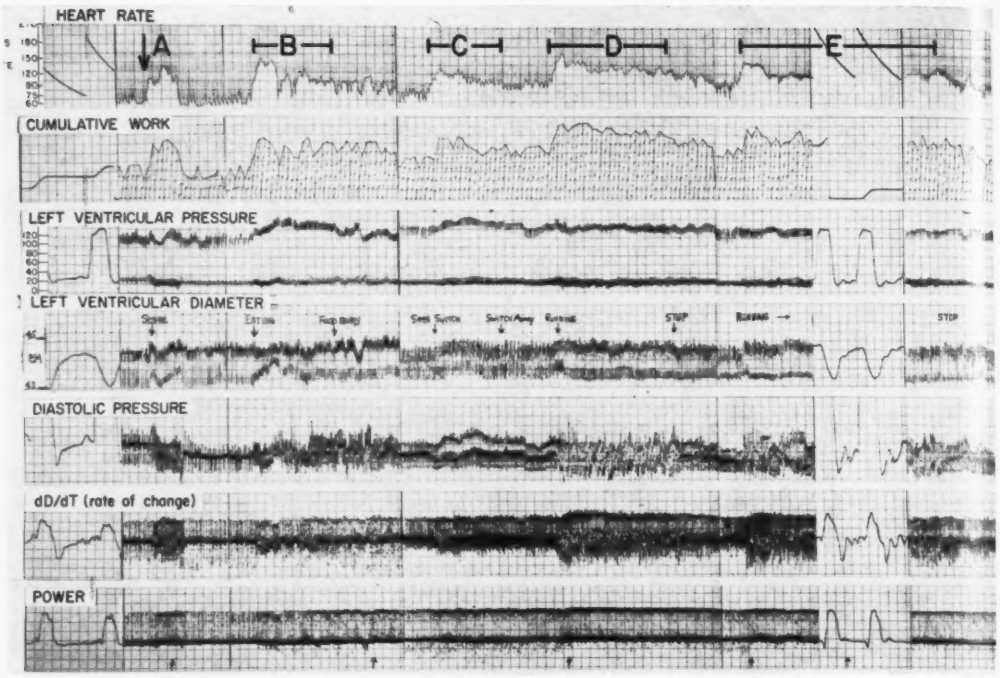

Figure 2

Changes in left ventricular performance of a healthy dog (28 days postoperative) under various conditions are illustrated; A, startle reaction from a loud noise; B, eating response; C, confronted with the switch that actuates the motor driven treadmill; D, exercise at 3 m.p.h. on a 5 per cent grade; E, repeat exercise with a record at high paper speed (50 mm./sec.) interposed in the middle of the response. Note that under many different circumstances, elevated systolic ventricular pressure is accompanied by tachycardia.

flow and cardiac output. The basic principles of this regulation are illustrated in figure 1. If the perfusion pressure is maintained constant by means of servo-control, any net change in peripheral flow is promptly countered by the appropriate increase in cardiac output. The distortion receptors in the carotid sinus and aortic arch could serve as appropriate sensory elements to monitor systemic arterial pressure. Theoretically, changes in the discharge frequency along the nerves from the pressoreceptors influence medullary cardioregulator centers, so that any reduction in arterial pressure would be promptly countered by the proper degree of tachycardia and compensatory vasoconstriction in inactive tissues to maintain systemic arterial pressure within a narrow range. The pressoreceptor mechanism has received a great deal of attention, since it is the only neural feed-back loop for cardiovascular control that has been described in some detail. Whenever this mechanism plays a dominant regulatory role, tachycardia should be associated with any reduction in mean systemic arterial pressure. Elevated systemic arterial pressure should be accompanied by bradycardia. On the contrary, cardiovascular responses of intact dogs during spontaneous activity frequently display tachycardia during elevation of systolic ventricular pressure (fig. 2). Other studies have indicated that these conditions are also frequently characterized by increased mean arterial pressure. Apparently the sympathetic discharge to the

heart under these conditions overrides the pressoreceptor mechanism. Under these conditions, the arterial pressure controls cannot be regulating cardiac output. Dr. Peterson will present additional information regarding this important mechanism.

According to traditional concepts, a mechanism for adapting the stroke volume to the circulatory requirements was embodied in the length-tension relationships of the myocardium, and no external control need be postulated. This mechanism is clearly dominant in isolated or exposed hearts under experimental conditions. Similarly, increased stroke volume and stroke work accompany increased diastolic distention in intact animals during the transition from the standing to the recumbent position.[1, 2] However, other forms of cardiovascular adjustments appear to be initiated primarily by increased discharge of sympathetic nerves to the heart and peripheral vasculature. In a dog that is familiar with treadmill exercise, confronting him with the switch that turns on the motor-driven treadmill will characteristically induce changes in left ventricular performance that simulate those produced by the running at 3 m.p.h. on a 5 per cent grade (fig. 2*C*). The left ventricular response to this treadmill exercise generally involves a prompt elevation in left ventricular systolic pressure accompanied by an equally abrupt tachycardia.[3, 4] In the particular example presented in figure 2, the systolic ventricular pressure was elevated when the treadmill switch was presented but not during the exertion. Thus an inappropriate stimulus produced a more typical response in figure 2*C* than did the appropriate stimulus in figure 2*D* and 2*E*. The left ventricular response to treadmill exercise at 3 m.p.h. on a 5 per cent grade can also be quite precisely duplicated by electrical stimulation in 2 discretely localized sites in the diencephalon (in the H_2 field of Forel and the periventricular gray matter). The response can be elicited by very weak stimulation without movement by the animals with or without anesthesia.[3, 4]

The initiation of changes in circulatory pattern by increased discharge from the higher levels of the nervous system does not necessarily constitute a regulatory mechanism under the definition presented by Dr. Peterson, since no sensing mechanisms, setting mechanisms, or feed-back loops have been implicated. (This is analogous to the occupant of the house resetting the thermostat.) Adjustments in the cardiovascular system initiated voluntarily or from higher levels of the nervous system might more appropriately be regarded as *control* rather than regulation. When a dog exercises for the first time on the treadmill, virtually all the recorded parameters tend to display an overshoot for 10 or 15 seconds and then settle down to a fairly constant level. However, by the third or fourth bout of exercise, this tendency to overshoot largely or completely disappears. This looks like modulation of an excessive cardiovascular response by unknown regulatory mechanisms.

Summary

If a regulating system consists of a mechanism for sensing the essential parameters, detecting deviations from a "set" value, and initiating corrective action, then the arterial pressure-regulating mechanism is the only regulating system described for the circulation in any detail. This mechanism appears to be important in maintaining a level of mean arterial pressure within a narrow range. It does not appear, however, to play a dominant role in adjusting the level of the circulation to meet spontaneously changing circulatory demands under many circumstances. During spontaneous activity by intact dogs, an increase in systolic ventricular pressure (and mean arterial pressure) is frequently accompanied by tachycardia as though some other mechanism were overriding the pressoreceptor mechanism. Thus, it appears that the circulatory system as a whole is largely controlled by neural mechanisms, usually initiated by higher levels of the nervous system. Very little is known of the sensory limbs of reflex arcs that can serve to regulate the cardiovascular system as a whole.

Summario in Interlingua

Si un systema regulatori pote esser definite como un mechanismo que percipe le parametros essential del processo a regular, que detege deviationes ab certe valores establite pro ille paramentros, e que initia actiones correctives quando tales es indicate, alora le mechanismo del regulation de tension es le sol systema regulatori in le complexo del circulation que ha essite describite in detalio. Iste mechanismo pare esser importante pro mantener le nivello medie del tension arterial intra strictissime limites. Tamen, illo non pare haber un rolo dominante in adjustar le nivello del circulation in responsa a spontaneemente alterate requirimentos circulatori sub varie circumstantias. Durante spontanee activitates per canes intacte, un augmento del tension ventricular systolic (e del tension arterial medie) es frequentemente accompaniate de tachycardia, de maniera que on ha le impression que un altere mechanismo se impone con vigor superior super le mechanismo pressoreceptori. Assi il pare que le systema circulatori in su totalitate es regulate in grande mesura per mechanismos neural que es usualmente initiate per nivellos plus alte del systema nervose. Pauchissimo es cognoscite del circuitos sensori o del arcos reflexe que pote servir a regular le systema cardiovascular in su totalitate.

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Regulation of Blood Vessels

By LYSLE H. PETERSON, M.D.

THE CARDIOVASCULAR system serves the biologic organism as a transportation and mixing system. The demand by the organism for this service varies in different tissues and at different times while the volume of blood in the organism tends to remain constant. The cardiovascular system fulfills its function, therefore, by varying the rate and the distribution of blood flow. Blood vessels play a major role in controlling the rate of flow of blood by affecting the resistance to flow, and they possess the entire responsibility for the distribution of the blood and its flow pathways. The primary aspect of blood vessels that affects the flow and distribution of blood is their caliber, i.e., their internal radius. This discussion will deal primarily with the factors that control the caliber of blood vessels and with the role of these factors in the regulation of the circulation of blood.

The concept of control of the caliber of blood vessels by the nervous system is relatively new. Moreover, the mechanisms by which the caliber of blood vessels vary as a result of nervous influences are only beginning to be understood and evaluated. Indeed, the youthfulness of many of our concepts of physiology and medicine is so striking that I cannot avoid mentioning it.

As late as 1831, Dr. E. Weber, a well-known German physician, said, "I believe that flushing and pallor could be explained if it could be taken for granted that the caliber of arteries becomes larger and smaller under nervous influence."[1] Only slowly has evidence begun to accrue during the past century that supports the idea of nervous control of the heart and blood vessels. It was not until after the Civil War that Ludwig and Cyon noted that stimulation of the central end of the aortic nerve causes bradycardia and hypotension, and it was 65 years later, in 1927, that Hering described similar effects following stimulation of the carotid sinus nerve. Subsequently, interest in the reflex regulation of the cardiovascular system expanded rapidly.

In the 1930's Heymans and his co-workers extended Hering's observations with a series of experiments that established that receptor areas lying outside of the central nervous system are sensitive to the behavior of the cardiovascular system and reflexly, through the central nervous system, control the behavior of the cardiovascular system in such a way that the behavior seemed to be regulated.

Also in the 1930's, Bronk became interested in these receptors and, with his colleagues, obtained records of the electrical activity of the nerve fibers arising from the carotid sinus, aortic receptors, and mesenteric Pacinian corpuscles. He stated, "It is their function to signal changes in blood pressure, and it is the function of their afferent fibers to provide a means of communication between these receptors and the central nervous system. Thus the centers are kept informed regarding the pressure within the blood vessels."[1] Heymans began a lecture[2] with, "For many years physiologists have been and are still occupied with the study of the mechanisms which regulate blood pressure. A precise and detailed knowledge of the regulation of pressure is the key, not only to the physiology of the normal circulation, but perhaps also to the pathogenesis of chronic arterial hypertension."

The concept developed quite naturally and has subsequently been widely held that *arterial blood pressure* is the function that is regulated through these receptors. This regulation of blood pressure is generally considered as an established principle of cardiovascular

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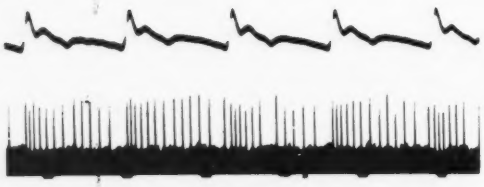

Figure 1

Electrical impulses from a single nerve fiber of a carotid sinus receptor together with a tracing of intraarterial blood pressure. (Reproduced by permission of the publisher and the authors from Bronk and Stella (1932).[1])

physiology. Figure 1 illustrates the well-known relationship between arterial blood pressure and receptor activity that Bronk and his co-workers found and that did much to establish the concept of blood pressure regulation.

Figure 2 is a schematic diagram, using the definitions stated in the introduction, which illustrates the concept of arterial blood pressure regulation. On the right of the diagram are the mechanisms that control blood pressure. Within each black box lies a mechanism that is considered to be a prime variable. The impulse traffic of the efferent nerves (I_e) arises from the central nervous system and commands, by mechanisms implicit in the black boxes, changes in peripheral resistance, stroke volume, and pulse rate. Thus, the product of stroke volume and pulse rate gives cardiac output, and the product of cardiac output and resistance gives blood pressure. These are among the elementary relationships that have been taught to every medical student for many years. In the top center of the diagram is the black box indicating that intra-arterial pressure causes a response in the afferent sensory mechanism ("pressure receptors") such that impulses of the afferent nerves travel to the central nervous system, thus transmitting information about pressure (I_p). On the left, the integrating mechanism, located within the central nervous system, is supplied with information regarding the level of blood pressure and in turn sends out efferent information in order to maintain the blood pressure at some ideal value—the "normal" blood pressure. Experimental work has established the apparent existence of each of these black boxes with the exception of that related to the establishment of an "ideal value" for pressure. Also, the organization of the black boxes into a closed loop that is linked together as shown seems reasonable. Thus, the concept of arterial blood pressure regulation seems to be established.

Many of the workers who have developed and perpetuated the concept of arterial blood pressure regulation realized that the so-called pressure receptors were not really stimulated by pressure directly, but rather by the stretching of the vessel wall as a consequence of intravascular pressure. They apparently thought, however, that vessel-wall stretch and intravascular pressure were related in such a manner that the 2 variables are equivalent. Furthermore, the concept of arterial blood pressure regulation may be considered to be a more general or important function, and a more easily measured function than vessel-wall stretch.

In the 1940's, some experiments directed attention specifically to the relationship of the vessel-wall stretch and the nervous output of receptors lying within the vessel wall. Hauss, Kreuziger, and Asteroth, in Germany, encased the carotid sinus with a rigid cement, thus preventing stretch of the carotid sinus when the blood pressure was increased, and thereby eliminated the reflex response to the increased pressure.[3] Also, Palme, working in Germany in the 1930's, applied epinephrine directly to the wall of the sinus and noted a marked fall in systemic blood pressure.[4] He also stimulated efferent fibers innervating the carotid sinus and obtained similar results. Palme concluded that the reflex effects obtained by him were due to direct stimulation of the receptors by the efferent nerves, although it might have been concluded that the efferent nerve activity altered the properties of the vessel wall and that this alteration, in turn, affected the stretch of the receptors for a given pressure.

Heymans[5] and Landgren[6] and their respec-

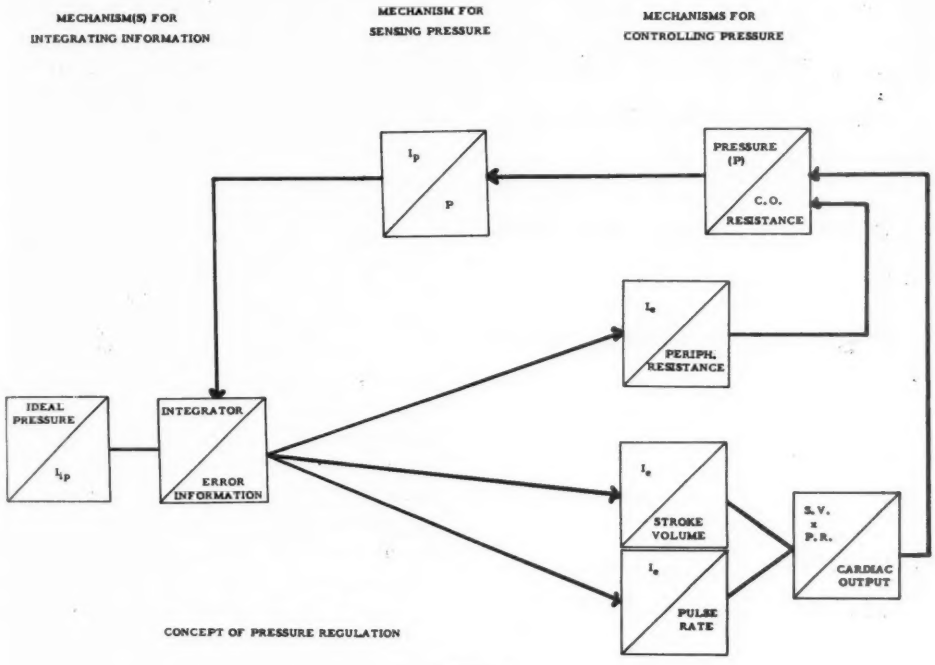

Figure 2

Schematic diagram representing the concept of blood pressure regulation. C.O. = cardiac output, S.V. = stroke volume, P.R. = pulse rate, I_e = efferent autonomic nervous information, I_p = afferent nervous information arising in the receptors and traveling to the central nervous system.

tive colleagues, and many others, have since applied a great variety of substances to the wall of the carotid sinus and the general conclusion has developed that the reflex changes that occur, but that are not due to blood pressure variation, are due to changes in the properties of the vessel wall, i.e., that the wall becomes more or less distensible. Heymans published a paper[5] in 1951 entitled, "New Aspects of Blood Pressure Regulation" and in the summary he stated, "These experiments show that the state of contraction and thus the resistance to stretch of the arterial wall where the receptors are located are the primary factors effecting the receptors which regulate systemic pressure."

During this period also many other characteristics of the receptors were discovered.[6] For example, it has been found that the traffic of nerve impulses arising in these receptors is a function of the rate of change of pressure as well as of the pressure itself. This implies that the pulsatile nature of arterial pressure is an essential aspect of normal cardiovascular regulation. This may be of importance in the use of extracorporeal bypass-pumps to maintain the circulation during heart surgery, since the normal pulsatile nature of the arterial pressure is reduced or absent.

Our definition of regulation states that there must be some mechanism for determining the magnitude of the function that is being regulated. The concept of blood pressure regulation can only be valid if there are receptors that measure pressure; yet it has been shown that the activity of these receptors is related to vessel-wall stretch and to the rate of change of stretch rather than to pressure and its rate of change. This distinction between stretch receptors and pressure receptors

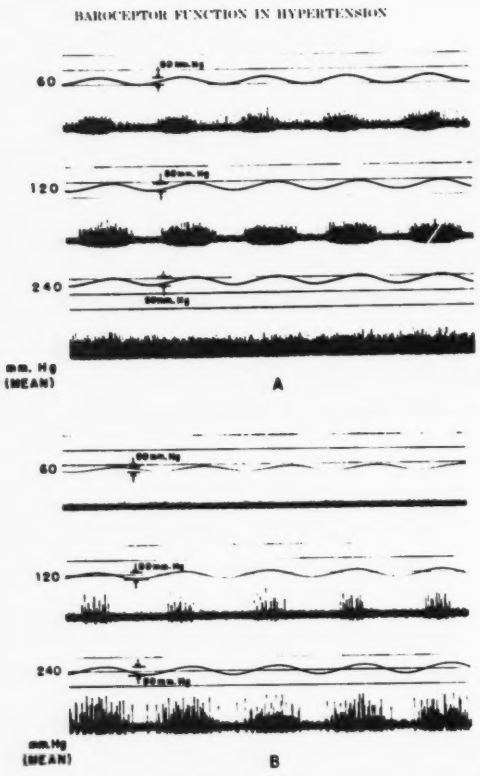

Figure 3

Neurograms from multifiber preparation of carotid sinus nerve of (A) *normotensive and* (B) *hypertensive dog during standard endosinus pressure stimulus. It can be noted that the activity of the receptors of the normal dog appears to be significantly less than that of the hypertensive dog although the pressure within the 2 sinuses is being made to vary in the same way. Breaks in top reference line at 1-second intervals. (Reproduced by permission of the publisher and the authors from McCubbin, Green, and Page (1956).[7])*

is of importance if the factors that relate vessel stretch and blood pressure are also variables under physiologic and pathologic circumstances.

The relationship between intravascular pressure and the circumference of the vessel wall is determined by the mechanical properties of the blood vessel wall. These properties are commonly referred to as vessel-wall tone or stiffness. It has been shown, as mentioned above, that these properties can be altered by placing vasoactive substances, such as epinephrine, acetylcholine, and norepinephrine on the carotid sinus wall and by stimulating the efferent nerves supplying the carotid sinus. The obvious question is, however, do these mechanical properties vary under natural circumstances? Certainly there is evidence that arteries become stiffer with age and in certain diseases. It is also known that most blood vessels of the body constrict and dilate under a variety of physiologic conditions, and it is not unreasonable to suppose that the receptor-containing vessels share in these changes. Moreover, it is known that in hypertension the properties of arteries (e.g., water and electrolyte content) change, there is vascular constriction, and the constituents of connective tissue may also change. Although there is every reason to suspect that many physiologic and pathologic conditions may affect the properties of the receptor-containing blood vessels, little research has been done in this regard.

One interesting series of experiments has, however, shown that the relationships between arterial blood pressure and traffic of the receptor impulses of the carotid sinus change during the development of renal hypertension. In 1955, McCubbin, Green, and Page[7] produced hypertensive dogs by wrapping their kidneys. Figure 3 shows representative data taken from one of their publications. Note that the receptor nerve-impulse output with respect to pressure is significantly reduced in the hypertensive animal as compared to the normal dog. Since this receptor activity acts, via the central nervous system, to tend to reduce blood pressure, it can be concluded that there is less depressor activity of the arterial blood pressure in the hypertensive animal than in the normal animal. Thus, the pressure could be expected to be increased in the hypertensive animal as a result of a change of carotid sinus-receptor behavior. It can also be concluded that this change in behavior is associated with changes in the mechanical properties of the sinus wall. It may also be

that in such hypertension states the integrating mechanisms of the central nervous system may be altered or the properties of the circulation control mechanisms may change. Indeed, the present medical director of the American Heart Association and his colleagues produced sustained hypertension in dogs by encasing the carotid sinuses in plastic jackets that restricted pulsation of the sinus wall.[8] Possible changes in cerebral blood flow associated with these studies, however, complicate the interpretation of the findings. In any case, the cardiovascular regulatory mechanism probably plays a vital role in the behavior of the cardiovascular system in health and disease. Clearly, however, the subject merits further investigation.

It is obvious that before this entire problem can be properly investigated and evaluated, it is necessary to understand and to be able to measure, quantitatively, the mechanical properties of blood vessel walls that relate the caliber or stretch of the wall and the intravascular pressure.

Recently methods have been devised for measuring these mechanical properties of blood vessels.[9] In brief, the principle can be illustrated as in figure 4. The uppermost figure represents a solid strip of material, e.g., a strip of blood vessel wall. T represents a tension stress (force per unit area) tending to stretch the material. l_o represents the initial length of the material before it is stretched and the shaded areas represent the increase in length that occurs when the tension is applied. Here the elongation of the material is a function of the amount of tension applied. But since the amount of elongation is also a function of the initial length of the material, the ratio of the increase in length to the initial length is measured instead of simply the total elongation. This ratio is called strain.

$$Strain = \frac{Change\ in\ length}{Initial\ length} = \frac{total\ \Delta l}{l_o}$$

The mechanical properties of this material that are of interest to us are those that determine the relationship between strain and stress, stress being the amount of tension applied to the material which in this case is related to the intraarterial blood pressure. If the material is purely elastic, i.e., has only the property of elasticity, then the relationship of stress to strain is given by

$$Stress = (E)\ (Strain)$$

in which E is called the modulus of elasticity,

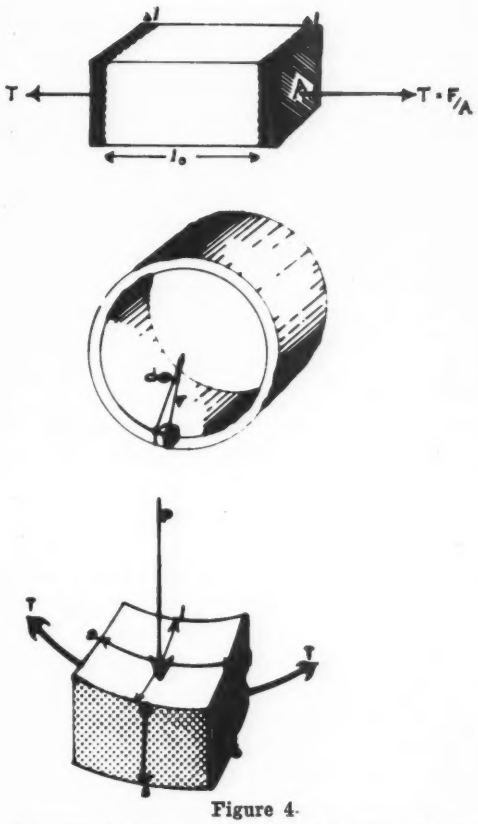

Figure 4

Uppermost *figure represents a solid strip of material. T = tension = force/area = stress applied to stretch, the material whose initial (unstretched) length is l_o. The change in length (Δl) results from the application of the stress. The* middle *figure is a cylinder analogous to a blood vessel. $d\theta$ is a small angle cutting a section of the wall. r is the radius of the cylinder. The small section cut from the wall of the cylinder is shown in the* lowest *figure. P = the pressure acting toward the wall, T = the tension tending to stretch the wall, a = the length of the arc, l = the length of the section, δ = the wall thickness. The relationship between T and P is $T = P(r/\delta)$.*[9]

the elastic stiffness or elastic resistance to stretch. Steel, for example, has a very high E whereas rubber has a much lower E. The E of arteries is similar to that of rubber.

Probably no real material is purely elastic. Certainly blood vessel walls are not purely elastic, rather they are viscoelastic. Viscosity is a quality often associated with molasses and is similar to friction; therefore, the blood vessel walls have, in addition to the elastic resistance to stretch, a resistance to stretch that depends upon how rapidly the stress is applied. Therefore, in the case of a viscoelastic material

$$Stress = E\ (Strain) + R\ (Rate\ of\ strain)$$

in which R is the modulus of viscosity. Materials also have the property of mass or inertia. It has been found, however, that, under living conditions, the effective inertia of blood vessels is negligible and does not appreciably contribute to their stiffness or resistance to stretch.[9]

The cylinder shown in the middle of figure 4 represents a blood vessel in order to illustrate the fact that the wall of a blood vessel is circular rather than lineal as shown above. Furthermore, it may be seen that the pressure acts radially, i.e., from the direction of the center of the cylinder, whereas the tension that stretches the wall is circular, i.e., in a different direction. The lower figure represents a piece cut from the wall of the cylinder to show the difference in the direction of the pressure stress and the tension stress. It can be shown that the 2 stresses are related by the radius of the cylinder and the wall thickness.[9]

$$Tension = Pressure \times \frac{Radius}{Wall\ thickness}$$

Thus, for the same internal pressure the stretching tension would be relatively small for a vessel of small radius and large wall thickness, and, conversely, for a vessel of large diameter and small wall thickness the tension would be relatively large for the same pressure. These relationships are important to an understanding of the properties of blood vessels, e.g., it is necessary to know the radius and wall thickness in addition to the pressure and strain if one wishes to compute the elasticity and viscosity of a blood vessel wall.

The often-used nouns, tone or stiffness of arteries can, therefore, be rigorously defined in terms of 2 mechanical properties, elasticity and viscosity. There are important distinctions between these 2 properties as they affect the stiffness of blood vessels. The elastic property is somewhat simpler in concept. It is analogous to the property of an ideal spring, in that its stiffness is independent of how rapidly it is stretched. The viscous property is analogous to viscosity of liquids; therefore, its contribution to stiffness does depend upon how rapidly the material is stretched. A viscoelastic blood vessel is therefore stiffer if pressure changed rapidly than if it changed slowly. Thus, the stiffness or tone of such a vessel would depend upon the rate of change of pressure during the cardiac cycle, i.e., upon the shape or contour of the pressure pulse.

The magnitudes and behavior of these properties of arteries in living, intact animals have been measured by simultaneously measuring the instantaneous intra-arterial pressure and vessel dimensions. The data were then analyzed by means of automatic computers. Thus, it has recently become possible to measure quantitatively and to define the "tone" or stiffness of arteries, which are their most important properties.[9]

Figure 5 illustrates, for example, the relationship between common carotid arterial diameter and pressure during each cardiac cycle before and after the application of norepinephrine to the vessel wall. Although the local application of norepinephrine did not change the systemic intravascular pressure, the average diameter and the pulsatile change in diameter which accompanies each pressure pulse cycle was reduced. This indicates a stiffening of the arterial wall caused by the application of norepinephrine. Analysis of such records has shown that norepinephrine can cause an increase in both the elastic and viscous stiffness of arteries by as much as 10 times.

Figure 6 illustrates the opposite effect that follows the application of acetylcholine to the wall of an artery. Here the magnitude of the average diameter and of the diameter pulsations are increased with respect to pressure, indicating a reduction in stiffness. Analysis has shown that, in arterial dilatation, although the elastic stiffness is reduced, the viscous stiffness is increased.

Such studies have emphasized several interesting characteristics of arteries under living conditions. It is sometimes thought that the diameter of arteries changes visibly and palpably during the cardiac cycle. For example, that palpation of the pulse is palpation of a changing vessel diameter. We have found, however, that the change in diameter, associated with a cardiac cycle, of even the largest and most distensible arteries of the body is very small. In most cases it is only 1 or 2 per cent of the average diameter. Thus, when one palpates an arterial pulsation, it is really the pressure changes that one feels rather than the dimensional changes of the vessel. Even though these latter changes are small, relative to the size of the vessel, their effect on the properties and behavior of the circulatory system is great. A slight change in diameter represents a larger change in the cross-sectional area of the vessel (area of a circle is proportional to the square of the diameter) and a change in the area produces a relatively larger effect on the resistance to blood flow. These small changes in diameter are also great enough to cause the receptors lying within the walls of blood vessels to have their characteristic discharge. It has been found that the elastic and viscous properties of arteries can undergo 10-fold variations and also, therefore, that the caliber and stretch of vessels undergo equivalent changes with respect to blood pressure.

Figure 7 is an illustration of the simultaneous measurements of intracarotid-sinus pressure, carotid sinus diameter, and carotid sinus receptor electrical activity that are being studied in our laboratory. Although these investigations are in a preliminary stage, they

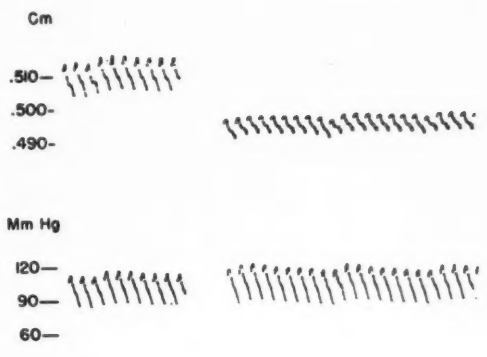

Figure 5

Relationships between intraarterial blood pressure and artery diameter at the same site. Upper *tracing is vessel diameter, calibration of diameter on far left;* left *section obtained during the control period,* right *section following the localized application of norepinephrine to the vessel wall.* Lower *tracing is intraarterial pressure.*

are being presented because they are pertinent to this discussion. The picture shows, from above downward, a continuous record of sinus diameter, intrasinus pressure, and electrical spikes from approximately 5 single receptor units, each having a different amplitude. On the left are the relationships before the application of norepinephrine to the wall. The strain is 0.038, i.e., the sinus is stretched 3.8 per cent during the cardiac cycle by the pulse pressure. Previously the stress-strain relationships that characterize the entire aorta and its major branches to vessels of 1 mm. in diameter have been studied. The strain or percentage stretch that the carotid sinus undergoes for a given increase in pressure, i.e., its distensibility, is considerably greater than that of any other arterial segment studied so far. As indicated in figure 7 the, pressure-elastic modulus (E_p) is approximately 1,500 Gm./cm.,^{2} which is only about 50 per cent of the average values of other arteries. It should be recalled that the distensibility of a vessel is a function of its radius and wall thickness as well as of its mechanical prop-

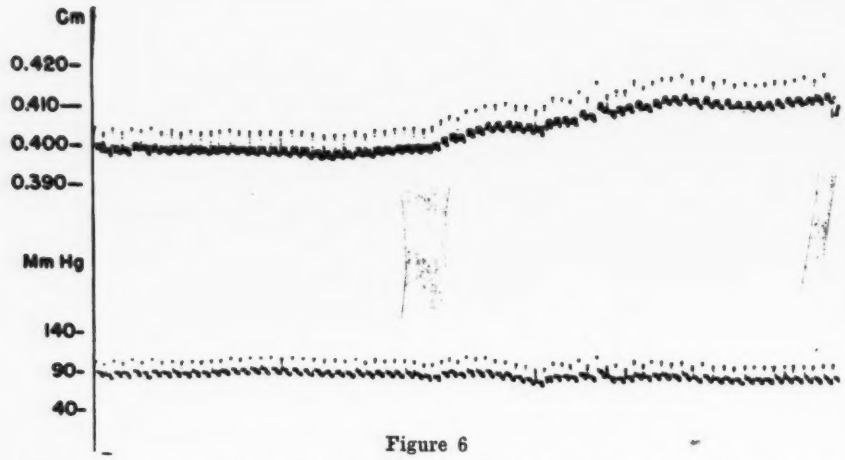

Figure 6

Comparison of vessel diameter (top) *and intraarterial blood pressure* (bottom) *at the same site (femoral artery). Acetylcholine was applied locally to the vessel wall. The resulting increase in diameter is obvious.*

erties. For example, it is known that the decreased distensibility that tends to accompany the increased stiffness associated with aging is often compensated for by an increase in its radius, i.e., dilatation. The low pressure-elastic modulus (high distensibility) of the carotid sinus is due primarily to the relatively high radius-wall thickness ratio. Indeed, the mechanical properties of the wall of the carotid sinus are quite similar to those of the aorta.

The records assembled at the right side of figure 7 were obtained following the application of a solution of norepinephrine to the wall of the carotid sinus. Although the intraarterial pressure pulses were not changed, the average and pulsatile diameter of the carotid sinus decreased. This indicates that the wall of the carotid sinus became markedly stiffer. The pressure-elastic modulus increased by 40 per cent, and, since the pressure pattern did not change, the strain decreased by 40 per cent.

These records show that marked changes can occur in the physical properties of the walls of the carotid sinus and that these changes can profoundly affect the activity of receptors lying in the vessel walls, which are independent of blood pressure changes but which, in turn, cause changes in the general cardiovascular system, including blood pressure changes.

The viscoelastic properties of the vessel wall are determined by a variety of tissue constituents, viz., smooth muscle, collagenous and elastic tissue, water, electrolytes, etc. It is most likely that physiologic control of the mechanical properties of the vessel walls generally is achieved by variations in the tension exerted by the smooth muscle in the vessel walls. Thus, the 10-fold variations in the elastic and viscous moduli found in the experiments recounted above is no doubt due to variations in the contractile force exerted by the smooth muscle. Moreover, it is also likely that chronic alterations in the properties of the vessel walls occur as a result of changes in the electrolyte and the water content of the vessel walls. Such variations have been reported to be associated with a variety of conditions including hypertension. It is possible, for example, that the renal hypertensive dogs studied by the Cleveland group suffered from alterations in vessel wall electrolyte and water content. Also it is known that alterations in the relative amounts of elastic and collagenous tissues accompany aging and certain diseases. It is also possible that the mechanical characteristics of the connective tissue itself may undergo changes.

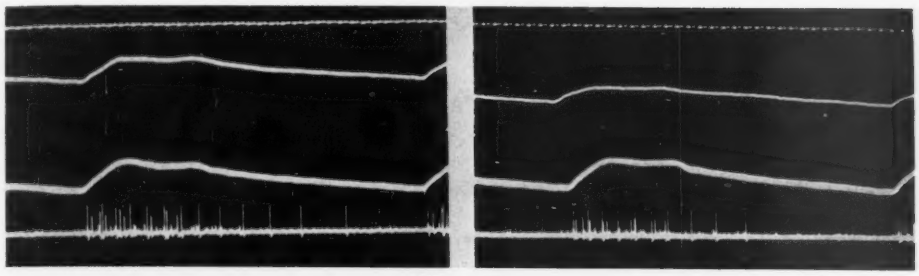

Figure 7

Comparison of carotid sinus wall motion (top tracing), *of intracarotid sinus pressure* (middle tracing), *and of the electrical activity of a few fibers of the carotid sinus nerve* (bottom) *before* (left) *and after* (right) *application of norepinephrine to carotid sinus, without change in intrasinus pressure.*

	Before epinephrine	After norepinephrine	
Strain (ϵ)	0.038	0.023	(40%↓)
Elastic modulus (E_p)	1492	2524	(40%↑)
Large spikes (av.)	13	10	(25%↓)
Small spikes (av.)	7	9	(24%↑)

Few, if any, of these factors that affect the mechanical properties of vessels have been adequately studied, and much remains to be learned before we can assess the role of vessel-wall properties in the regulation of the circulation. It is likely that these properties will prove to be internally related to the regulation of the circulation.

We may summarize the factors affecting receptor activity as follows: 1. Receptor stimulus is probably the strain of the vessel wall in which the receptors are located. 2. The strain is a function of at least 4 biologic variables, namely, intraarterial pressure, rate of change of intraarterial pressure, elastic modulus of the vessel wall, viscous modulus of the vessel wall. 3. Any given receptor area, e.g., the carotid sinus, contains a population of receptors of which each receptor may have different thresholds or sensitivities, and perhaps some may respond differently with respect to the direction of the strain. The information that is significant to the central nervous system may be contained in the net traffic from entire populations of receptors from a variety of areas.

It is obvious that much remains to be learned about these factors and their interrelationships. It is also obvious that the concept that pressure per se is regulated is too simple and may have been misleading.

So far I have mainly discussed the receptor mechanism of the carotid sinus as an example of the manner in which regulation of the circulation may be considered. Many other "mechano-receptor" areas have been described, but the carotid sinus mechanism has been most widely studied and therefore more information about its function has accumulated. It is impossible to say, however, what the relative role of this and the numerous other receptor areas may be in the over-all regulation of the cardiovascular system. Aviado and Schmidt[10] have reviewed studies of a variety of receptor areas that are known to affect the cardiovascular and pulmonary systems. In addition, there are probably many receptor areas yet to be discovered.

A number of receptor areas within the vascular system have been called volume-receptor areas in contrast to these other areas referred to as pressure-receptor areas. These so-called volume receptors have been found particularly in the great veins and pulmonary vessels and, when stimulated, may cause reflex variations in cardiovascular, pulmonary, and renal functions. Dr. Elkinton will comment upon the fact that such receptors play an important role in the control of the body's water and electrolyte content. In reality, the so-called pressure and volume receptors and their activity is a function of the intravascular pressure and the mechanical properties of the vessel walls that

contain the receptors. The difference in the names probably arose because in the systemic arteries pressure changes are large compared to volume changes, whereas the volume changes of the systemic veins and pulmonary vessels are large compared to pressure changes. Thus, the major factors responsible for the difference are the mechanical properties of the blood vessels.

It is instructive to consider how many functions of the cardiovascular system are affected by the mechanical properties of the blood vessels. As stated above, they affect (1) the blood pressure and blood volume, and (2) the activity of receptors located within blood vessel walls, as well as (3) the vascular resistance to flow by producing vasoconstriction and vasodilatation. They affect (4) the performance of the heart by altering the venous return and the arterial load. By their effect on blood pressure they affect (5) the exchange of fluid between the intravascular and extravascular fluid spaces. They (6) direct the flow of blood through various vascular pathways. They affect (7) renal function both through neuroreceptor mechanisms and by their controlling effect on renal blood flow. The mechanical properties of the blood vessels, therefore, must play a predominant role in the regulation, not only of functions of the cardiovascular system, but also of the entire, integrated regulation of the biologic functions of the body.

An interesting interrelationship exists between the activity of strain receptors located within the vessel walls and the mechanical properties of the vessel walls. The activity of the receptor is a function of the mechanical properties of the wall, since those properties determine the amount of strain that results from a certain amount of stress (e.g., pressure). In turn, the reflex response associated with this receptor activity results in a change in the mechanical properties of the vessel walls, i.e., this is the mechanism by which the caliber of blood vessels is controlled. This situation is similar to what would occur in the temperature regulation of the house, talked about in the introduction of this symposium, if the thermostat were so made that whenever the temperature within the house changed, an alteration of the "setting" of the thermostat would occur. The extent of this effect as well as its significance is unknown but again is an indication of our naivete regarding regulation of the cardiovascular system.

I have said little regarding the important aspect of cardiovascular regulation that is associated with the central nervous system. This has been lumped into the integrating and error-generating black box in figure 2. Dr. Rushmer has shown us that voluntary or conscious central nervous system activity can duplicate the cardiovascular responses to exercise. This, it seems to me, is an excellent example of the influence of these higher centers upon the regulatory mechanisms that may be analogous to the resetting of the thermostat by the occupant of the house mentioned during the introduction of this symposium. There is little doubt that the cardiovascular system is controlled during exercise, but at a higher level of activity. It is likely, however, that there are other factors in addition to the drive of the higher central nervous levels in establishing the "ideal" or "set" values about which regulation takes place. Indeed, the role of the central nervous system in the regulation of the functions of the cardiovascular system is still largely unknown. Some of these questions will be discussed by Dr. Folkow.

I have tried to show, in this brief period, that in considering the regulation of blood vessels there are vast areas that are poorly understood and, indeed, that fundamental concepts of what is regulated and how regulation occurs are not understood. It is evident that the diagram shown in figure 2 is woefully inadequate and that even the functions of the black boxes shown therein are not well understood.

It is not surprising, therefore, that the etiology of hypertension has escaped detection or that we cannot explain the physiologic responses to stresses such as exercise. The surface of cardiovascular physiology has hardly been scratched, and there are many fundamental, challenging problems facing investigators.

While the advances of the past 300 years

may seem disappointingly few, there is reason for optimism. The next decade may bring greater advances than those of the entire past 3 centuries. It has been said that more than 80 per cent of all the physiologists who have ever lived are now alive. It is evident in most phases of human endeavor that the rate of achievement of knowledge is, like population growth, a rapidly accelerating phenomenon.

Addendum

Two recently published books, to which the reader may wish to refer, have come to the author's attention since this discussion was prepared.[11, 12]

Summario in Interlingua

Le systema cardiovascular servi le organismo biologic como systema de transporto e de mixtion. Le requirimentos del organismo pro iste servicio varia in differente tissus e a differente tempores durante que le volumine de sanguine in le organismo tende a remaner constante. Ergo le systema cardiovascular executa su function per variar le intensitate e le distribution del fluxo de sanguine. Le vasos de sanguine ha un rolo major in determinar le intensitate del fluxo de sanguine in tanto que illos exerce un influentia super le resistentia contra le fluxo. Le vasos de sanguine ha le complete responsabilitate pro le distribution del sanguine e le circuitos de su fluxo. Le aspecto principal del vasos de sanguine que affice le fluxo e le distribution del sanguine es lor calbre.

Le presente articulo summarisa le recercas effectuate relative al factores que determina le calibre del vasos de sanguine e al rolo de iste factores in le regulation del circulation del sanguine. Le discussion concerne primarimente le mechanismo receptori del sino carotidic que es presentate como exemplo del maniera in que le regulation del circulation pote esser considerate.

Le autor formula le conclusion general que in le campo del regulation del vasos sanguinee il existe vaste areas que es mal comprendite a iste tempore e de facto que conceptos fundamental con respecto a qual processos es regulate e a como le regulation occurre es non ancora sufficientemente clar.

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Role of the Nervous System in the Control of Vascular Tone

By BJÖRN FOLKOW, M.D.

IT IS well known that changes in vascular smooth muscle tone form the most powerful tool for the control of blood flow changes. Though it is generally agreed that the nervous factor is the most important one, it still is an open question how many, and which, mechanisms contribute to smooth muscle tone. An attempt will therefore be made to outline briefly the classical concepts of vascular control as contrasted to our present views, especially the relation of nervous control to other important mechanisms.

It is useful to define a baseline for activity of vascular smooth muscle. Such a baseline is best defined as the hemodynamic state created when vascular smooth muscles are at complete rest. This is most conveniently measured in terms of the flow resistance per 100 ml. of tissue at a normal distending and perfusing pressure, when a state of maximal vasodilatation is induced.[1] The level of vascular tone can then be quantitatively expressed as the ratio between the actual and the minimal flow resistance, just as one can estimate the state of activity of any isotonically contracting muscle from the extent of its active shortening. Of course this implies many simplifications but it is useful and easily performed.

This minimal flow resistance varies markedly in different tissues, which is not surprising since the respective vascular circuits can be expected to be structurally tailored to meet the extensive metabolic variations that are known to occur from one tissue to another. When expressed in terms of ml. flow/min./100 ml. of tissue the approximate values are, for example, for the myocardium 300 to 400 ml., for skeletal muscles 50 ml., for the brain 100 to 120 ml. In some tissues one finds values far higher than needed to satisfy maximal nutritional needs, e.g., in the skin, most glands, and the kidneys. Here, however, blood flow also serves other purposes: in the skin to allow for increases of heat loss and in glands and the kidneys to supply the raw materials necessary for secretion and urine formation.

The maximal blood flow capacity is, of course, only infrequently utilized; the vascular circuits are normally dynamically restricted by the vascular smooth muscles, the average activity level of which we call "vascular tone." This vascular tone implies the "blood flow reserve" of the tissues and it can be mobilized by smooth muscle inhibition. We come then to the basic questions in vascular regulation: exactly which mechanisms build up vascular tone and which factors are responsible for its inhibition?

These questions have been studied for more than 100 years, but we are far from the end of the story. Perhaps it is best first to outline the "classical" concepts. The main principles of vascular control were outlined by the pioneer studies of the nineteenth century that Bayliss[2-4] coordinated to form a hypothesis, which was widely accepted for decades to follow. Being struck by the reciprocal control of the heart and the somatomotor neurons, he suggested that the blood vessels should also be under a reciprocal tonic nervous influence. The vasoconstrictor fibers and the hormones of the adrenal medulla should constitute the excitatory link under control of a vasoconstrictor center, in tonic reciprocal balance with a vasodilator center, controlling the vessels via sympathetic, parasympathetic, and dorsal root dilator fibers. This nervous control was thought to be fairly diffusely organized, executing mainly mass activation and inhibition patterns. It was also often assumed that what remained of vascular tone after cutting the

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vasomotor nerves, should be mainly a consequence of constrictor agents in the blood. Lastly, though the vasodilatation in activated tissues was known to be mainly due to local factors ("vasodilator metabolites"), neurogenic vasodilator influences were often thought to form an important contribution. This gives in rough approximation the classical concept of blood vessel control, and we will next consider the question as to how the research of more recent years has changed these views.

It is now clear that the vasoconstrictor fibers form the sole efferent nervous link for tonic vascular control. In fact, the most frequent type of neurogenic vasodilatation is caused simply by inhibition of the prevailing activity of the medullary vasoconstrictor fiber center (VMC). There is no evidence of any tonically active, common vasodilator fiber center and the 3 vasodilator fiber types do definitely not form any functionally homogeneous group.[5–7]

It might be best to start by outlining the functional significance of the vasodilator fibers and return to the more important, the vasoconstrictor fibers, later. The sympathetic cholinergic vasodilator fibers are distributed only to the vessels of the skeletal muscles and possibly also to the coronary vessels. These fibers appear to be activated under circumstances in which they act in *synergism* with the vasoconstrictor fibers rather than being in reciprocal balance with them. Their highest representation is found in the vicinity of the motor cortex of the central nervous system, with relay stations in the anterior hypothalamus and in the mesencephalon, where the third order neurons bypass the VMC to make direct contact with the "final common path" in the lateral spinal horns, i.e., the preganglionic sympathetic neurons. On topical excitation of the hypothalamic integration station vasodilator fiber activation is combined with an excitation of vasoconstrictor fibers running to the skin, the gastrointestinal tract, and the spleen. In addition, activation of accelerator fibers to the heart and a hormone discharge from the adrenal medulla occurs. With very little shift in arterial pressure this autonomic activation pattern leads to a remarkable and instantaneous redistribution of the cardiac output to favor the skeletal muscles. When this region of the hypothalamus is topically stimulated in awake animals, a behavior typical of spontaneous rage or fear is induced, i.e., the autonomic pattern appears to be normally combined with a quite characteristic somatomotor activation pattern.[8] Though these data are most suggestive, we cannot say that it is exactly known under which circumstances these fibers are normally activated. It is reasonable to assume activation in what Cannon called "emergency situations," simulating reactions of attack, defense, or flight. Furthermore, it is not excluded that these fibers might be more generally activated at the onset of muscular work, thus anticipating the needed increase of blood supply, though there is no experimental evidence. What is clear, however, is that the sympathetic vasodilator fibers are not engaged in the tonic control of the blood pressure as exercised reflexly from the different sets of cardiovascular proprioceptors.

The parasympathetic vasodilator fibers were previously thought to be widely distributed, for instance, in the gastrointestinal tract. It now seems that the majority of the so-called parasympathetic vasodilator fibers are not specific dilator fibers. On stimulation of the parasympathetic supply of the salivary glands, for instance, there is a release of a proteolytic enzyme, which splits off a polypeptide from normally present proteins in the tissue fluid.[9] This polypeptide, often called bradykinin, is closely related to, or even identical with, pancreatic kallikrein, described thirty years ago, which also has a profound vasodilator action.[10] It appears likely that a similar substance is released also from other activated gastrointestinal glands, and bradykinin is definitely released from the sympathetically innervated sweat glands, explaining the intensified cutaneous vasodilatation occurring when sweat production starts.[11]

The remaining specific parasympathetic vasodilator fibers appear to be those running to the pial vessels and the external genital

organs. Virtually nothing is known about the functional significance of the first-mentioned type going to the pia; they might, for instance, be activated in arousal stimulations from the reticular formation, thus contributing to increasing cortical blood flow. If so, they are not unrelated to the sympathetic vasodilator fibers with respect to the circumstances that excite them. But this is only speculation, and one feels more certain about the functional significance of the vasodilator fibers to the external genital organs.

The dorsal root vasodilator fibers, which Bayliss thought to convey efferent vasodilator impulses, are truly afferent fibers with no normal efferent function. It is known[12] that the periph ral ramifications of pain fibers form an axon reflex arrangement with the adjacent blood vessels, responsible for the "flare" in "triple response." In all probability dorsal root vasodilatation is simply due to the antidromic activation of this pain fiber axon reflex arrangement. On antidromic excitation the stimulation strength has to be increased enough to excite also the C-fiber group before vasodilatation appears. The majority of the pain fibers belong to the C-fiber group and are distributed to superficial tissues such as the skin and certain mucous membranes. It is only within these tissues that any significant vasodilatation is obtained on dorsal root stimulation. An arrangement of this type must be of some value as a local defense mechanism against harmful stimuli, since regional blood flow is automatically increased as soon as pain fibers are excited. Since such fibers are also activated when the skin is chilled more intensely, it is not surprising that the axon reflex mechanisms appear to form one of the factors that are responsible of cold vasodilatation in the skin.

It is evident that these 3 sets of vasodilator fibers are neither tonically active, nor do they form any homogeneous group, but are specially engaged only under quite specific circumstances. The steady tonic neurogenic adjustment of the vascular bed is, as mentioned, exercised only by the vasoconstrictor fibers, and are engaged in practically any sort of cardiovascular adjustment, which make them the most important vasomotor fiber type. This does not mean, however, that they are the *only* important factor for the establishment of vascular tone. When they are regionally blocked, there remains in many tissues a marked vascular tone that we can call *basal tone*. For instance, in resting skeletal muscles blood is normally around 3 to 5 ml. per 100 ml. of tissue per minute, increasing to roughly twice the value on regional vasoconstrictor fiber block. It can, however, be increased another 4 to 6 times, up to about 50 ml., in muscular work. This implies a pronounced basal tone in this tissue. This is also the case in many other tissues, e.g., the myocardium and the brain; after elimination of all possible vasoconstrictor fibers to these tissues their vessels still maintain a considerable tone, though it is promptly inhibited by increased tissue activity. As a contrast to this, vessels like the cutaneous arteriovenous anastomoses become practically maximally dilated as soon as their constrictor fibers are cut, provided that the organism is in a "resting" equilibrium without any significant reflex increase of the hormone output from the adrenal medulla.[1, 7]

What is then the background of this basal vascular tone, which in some areas is so pronounced, in others moderate, and in still others practically absent? If it were due to blood-borne vasoconstrictor substances one would expect that the vessels with high basal tone should be *more* sensitive to such factors than, e.g., the cutaneous arteriovenous anastomoses. Exactly the opposite is in fact the case; for example, the vessels of the skeletal muscles are definitely *less* sensitive to all known biogenic vasoconstrictor substances than are the cutaneous arteriovenous anastomoses, which respond to exceedingly low blood concentrations. From these simple observations one can conclude firstly that basal vascular tone must be of an essentially local origin, secondly that in the normal resting organism the blood concentration of vasoconstrictor agents is fairly negligible.[13] In fact normally the blood concentration of the hor-

monal component of the sympatho-adrenal system exerts more significant excitatory effects on heart and the blood vessels only episodically and are then generally overshadowed by the direct sympathetic innervation.[14] With exception of such episodic conditions and some pathophysiologic circumstances, as when angiotension is released via renin production, there is no evidence of functionally significant concentrations of vasoexcitor substances in the blood.

There is, on the other hand, good evidence that vascular smooth muscle cells, especially those of the smallest vessels, adjacent to the capillaries, show a rhythmic inherent activity, justifying the term myogenic automaticity. This is a well-known phenomenon in some other types of smooth muscles, but it is in some specialized types virtually absent, as in the intrinsic eye muscles.[15, 16] A myogenic activity will, of course, be profoundly influenced by external factors, but the essential point is that it is not basically a matter of any extrinsic excitatory influence, rather it is dependent on a normal environment, as are all forms of cellular activity. If great numbers of vascular smooth muscle cells show rhythmic but unsynchronized contractions, this will create a "vascular tone," and it is reasonable to assume that this is the very background of what has here been called basal tone. The myogenic activity, and hence the basal tone, is certainly not equal in all types of small vessels. The smooth muscles of the cutaneous arteriovenous anastomoses resemble the intrinsic eye muscles, both in that they are normally quite dominated by the central nervous system via the autonomic nerves and that these specialized smooth muscles show very little myogenic activity and consequently little basal vascular tone.

In 1902, Bayliss[2] offered a hypothesis suggesting that the distention offered by the blood pressure may act as mechanical stimulus on vascular smooth muscles. For decades this interesting idea has been almost forgotten, and for the first time in recent years it has been possible to show that this is a factor to consider,[17–19] though its influence is far from dominant. It has been difficult to understand exactly how such a mechanism operates, but now a reasonable explanation may be offered. It is known from studies of nerve-free intestinal smooth muscle strips, that distention has a facilatory influence on the rhythmic, inherent activity, increasing the *rate* of contractions.[16] This may also happen in those parts of the vascular bed where the smooth muscles exhibit myogenic activity. In fact, it has been observed in the vessels of the bat's wings,[20] where the rate of rhythmic "vasomotion" has been observed to increase as a response to steady distention of the vessels. This should mean that the time fraction occupied by the constriction phase increases on the expense of relaxation, and the integrated result of this in a vascular bed is a somewhat increased tone within those sections where myogenic activity occurs. Also, it is then understood why vessels like the cutaneous arteriovenous shunts do not increase their tone as a response to distention; these centrally dominated vessels hardly show any myogenic activity that could be facilitated.

The origin of the "basic activity" of the vascular smooth muscle cells has been discussed, now we will concentrate our interest on the powerful, superimposed influence of the vasoconstrictor fibers. Vasoconstrictor fiber distribution shows a considerable differentiation, with a tendency of an inverse relationship between the extent of basal tone and constrictor fiber supply. To mention the extremes, the heat-loss regulating cutaneous arteriovenous shunts, which serve no local needs but the organism as a whole, are supplied with an abundance of constrictor fibers but have, as mentioned, hardly any basal tone. In contrast, vessels subserving the metabolic needs of vitally important tissues, like the brain or the myocardium, have only few constrictor fibers but nevertheless a considerable "blood flow reserve" in the form of their locally controlled basal tone. Between these extremes the other vascular circuits are found. In generalized sympathetic discharges, as found with hemorrhage, this distribution will have the consequence that the reduced

cardiac output will primarily be delivered to the vital, important tissues.

It should be remembered that effector cells can exhibit various degrees of sensitivity to the constrictor mediator in different vascular areas. It has already been mentioned, for instance, that a given norepinephrine concentration has a stronger effect on the cutaneous arteriovenous shunts than on the vessels of the skeletal muscles.

It is also of importance that the vascular smooth muscles appear to vary in their sensitivity to locally produced vasodilator influences. This factor also determines the vascular response to constrictor fiber activation, as the reduced flow leads to an accumulation of vasodilator factors which counteract the effect of the constrictor fiber mediator. Differences in smooth muscle sensitivity can be strikingly illustrated if one compares muscle and cutaneous vessels. Lastly, the wall-to-lumen ratio of the vessels influences the hemodynamic result of a given sympathetic vasoconstrictor fiber activation.

What then is the influence of the main physiologic variable, the extent of vasoconstrictor fiber recruitment and its discharge range? It is obvious both from the classical neurophysiologic studies of Bronk et al. some 20 years ago and from other types of experimental approaches,[6, 7] that the physiologic discharge rate of visceromotor fibers hardly ever exceeds about 10 impulses per second, even under extreme excitation of the centers. Higher frequencies are, in fact, hardly needed because the almost maximally obtainable effector response to nerve stimulation is then reached. "Normal" vasoconstrictor tone appears to be maintained by a joint activity of most fibers to a given vascular area, firing at rates of only 1 or 2 impulses per second. If one compares the visceromotor neuroeffectors with the somatomotor ones, there is a fairly good 1-to-10 relationship in fiber diameters, refractory period, physiologic discharge range, and frequency response characteristics of the respective effector cells.

As mentioned earlier, the hormonal sympathetic link has, in most areas, fairly insignificant excitatory effects on the cardiovascular system when compared with the direct vasoconstrictor innervation. The situation is quite different for the *inhibitory* influences, i.e., the vasodilatation induced by blood-borne epinephrine in tissues like the myocardium and the skeletal muscles. Even the minute blood concentrations obtained at low-frequency fiber discharge, can elicit rather powerful vasodilatations, and this reaction is normally the most important cardiovascular effect of the hormonal link on generalized sympathetic excitation, i.e., it may contribute significantly to divert the cardiac output to the muscles and heart.[14, 21] That the hormonal link is of relatively insignificant importance in many respects is hardly surprising; its diffuse and relatively slow action would otherwise make rapid and differentiated cardiovascular adjustments impossible; such adjustments must be executed by a dominating, direct nervous link. In this area some very important questions concerning the nervous control of the vessels are raised: 1. Do similar differentiations in fiber distribution, etc., occur between the different *consecutive* sections of a vascular circuit? 2. Are there also strictly regional differences in discharge rate, and, if so, how extensive? Earlier the sympathetic nervous system was mainly looked upon as a sort of mass activity system and, in some circumstances it is; but this does not exclude the possibility of differentiated reaction patterns. To deal with this aspect it is also advisable to divide the effector system, i.e., the vascular bed, according to its functional specialization. In the first line one can distinguish between the various *"parallel-coupled"* circuits, e.g., coronary, cerebral, renal, muscular vessels. As already indicated, they often show considerable differences in fiber distribution, effector sensitivity, etc. Further, any of these parallel-coupled circuits can be divided in functionally defined "series-coupled" sections: (1) *"Windkessel" vessels,* (2) *resistance vessels* with a precapillary and a postcapillary section, (3) *precapillary sphincters,* which determine the number of capillaries, and hence the capillary surface, open for perfusion a

any given moment, (4) *shunt vessels* and, (5) *capacitance vessels.* All these sections, controlled by smooth muscle cells, are responsible for well-coordinated but essentially separate control functions, all of which, directly or indirectly, cooperate to control the flow through the most important section of all, the true capillaries. Across the walls of these "exchange vessels" the tissues come in close contact with the mobile fraction of the stable environment. Quite a bit is known about the resistance vessels, much about the capillaries,[13] and some about the shunts, at least the ones in the skin. Relatively little and, in some respects, nothing is known about the range of control of the other sections and their integration with the rest of the circulation. This is mainly because they are far more difficult to study separately with more exact methods. These vascular sections are, however, functionally just as important as the better-studied ones. Changes in tone of the capacitance sections, for instance, will profoundly affect the venous return to the heart and hence the cardiac output. Several attempts have recently been made to develop methods allowing more detailed analyses of the integrated control of capacitance vessels in relation to resistance vessels and heart function.[23–25] In our laboratory a method has been used,[27] which is somewhat related to the one utilized by Pappenheimer et al. in their classical capillary studies.[22] Our method makes it possible to follow continuously and separately the range of nervous and hormonal control of the precapillary and postcapillary resistance vessels, the capacitance vessels, and to some extent the precapillary sphincters and the cutaneous shunts. The results illustrate that the vasoconstrictor control of the capacitance vessels in the cat's hindbody in some respects is even more powerful than that of the resistance vessels. The hyperbolic curve, relating stimulation frequency to effector response, for the capacitance vessels is displaced to the left of that of the resistance vessels, i.e., at a slight generalized increase of constrictor fiber discharge the "intravascular fluid mobilization" is relatively more pronounced than the increase of flow resistance. The constrictor fiber influence on the 2 sections of the resistance vessels is, generally, more intense on the precapillary section than the postcapillary section, which implies that mean capillary pressure decreases on sympathetic stimulation even if arterial inflow and venous outflow pressures stay the same. This leads to a shift in the Starling equilibrium across the capillary walls, so that tissue fluid passes into the blood stream. The constrictor fiber control of the resistance vessels therefore implies possibilities of an "extravascular fluid mobilization" to the vascular compartments. Lastly, the sphincter sections are to some extent influenced by constrictor fibers, though not so markedly. There occurs an unquestionable decrease in capillary surface area available for perfusion, presumably because constrictor fiber activation causes a fraction of the precapillary sphincters to obstruct flow through their respective capillaries.

Results indicate that the sympathetic vasodilator fibers to skeletal muscles act almost only on the resistance vessels. Epinephrine, which increases blood flow to the muscles, generally also decreases the blood volume within the muscles, i.e., while it inhibits tone in at least some section of the resistance vessels, it excites the smooth muscles of the capacitance vessels. Further, although norepinephrine strongly contracts both resistance and capacitance vessels, angiotensin has a profound action only on resistance vessels. All these data illustrate differentiations between the different "series-coupled" vascular sections concerning vasomotor fiber distribution and effector sensitivity to transmitters as well as other vasoactive substances.

We then come to the second question: Can, by way of higher centers or cardiovascular receptors, the neuron pools controlling the various parallel, and the series-coupled vessels be more or less separately activated? If so, the nervous control of the vascular bed is indeed far from a diffuse network fitted only for mass activation patterns, but a highly specialized system suited for considerable degrees of differentiation. There is some evi-

dence in the literature, that such differences do occur between the various parallel circuits though much remains to be done; e.g., the constrictor fiber discharge to the renal or the cutaneous vessels is often not the same as that to other vascular circuits. So far, the series-coupled sections have been inadequately studied with suitable methods to allow any definite statements. However, experiments are going on with the mentioned method, in which the vasomotor fiber connections to the studied region are left entirely intact. Under such circumstances the normal discharge patterns, induced from cardiovascular receptors or higher vasomotor centers, will affect the different consecutive vascular sections of the cat's hindbody. Though they are undoubtedly fairly equally affected in many reaction patterns, some preliminary observations suggest that striking differentiations in the discharge patterns may also occur under some circumstances. It should be admitted here that the more detailed functional analysis of the autonomic nervous system has been badly neglected, when compared with the somatomotor system. It may ultimately prove to be just as differentiated in its neuroeffector control. Even if it should be found that great differences in regional vasoconstrictor fiber discharge do not occur, it should be stressed that the responses of the effector cells in different vascular sections may nevertheless be strikingly different, simply because of differences also in fiber distribution, effector sensitivity, etc.

A few words should be said about the higher centers controlling the vasoconstrictor fibers. It is well known that the medullary vasomotor center (VMC) is a very capable reflex center, responsible for the tonic control of blood pressure. It is, however, normally continuously modified by the activity of diencephalic and cortical autonomic centers. No details about their organizations will be given here, for which the reader is referred to other review articles,[6, 7, 27] but a few principles should be mentioned. For decades the higher centers of cardiovascular control were more or less neglected, and many investigators assumed that neurogenic circulatory adaptations as in muscular work, were predominantly started in the periphery by way of the cardiovascular proprioceptors and VMC rather than from the cortex-diencephalon. It might be, however, that the VMC is only one part of the system, a sort of autonomic equivalent to the somatomotor "antigravity center," and that the higher autonomic centers have numerous direct connections with their "final common path," the preganglionic cells of the spinal medulla. It becomes more and more clear, and it should have been obvious long ago, that the higher centers generally act as the initiators of cardiovascular adjustments that accompany changes in alertness and the onset of muscular work. The autonomic activation patterns probably run parallel with the somatomotor ones, thus "anticipating" the cardiovascular shifts needed to suit the change in activity. In all probability, however, this central direction of the cardiovascular system is not too exactly balanced, but becomes so when it is automatically adjusted by the cardiovascular receptors via VMC, so that the ultimate pattern results in small changes in pressure and is well regulated to the new level of muscle activity. We may here draw a parallel to the respiratory control, in which a similar interplay between central direction and reflex medullary fine-adjustment appears to take place. Concerning central cardiovascular control, however, our knowledge is still meager indeed but it is a fascinating field of research. Mention only is needed of the hypothalamic temperature control of cutaneous blood flow, the cardiovascular shifts taking place in emotional fainting, anger, fear, and muscular work to indicate the importance and interest it deserves. Its clinical implications are obvious; one need only mention so-called psychosomatic disturbances.

After this short survey of the factors that establish vascular tone a few words should be spent on the factors that *inhibit* vascular tone, the so-called vasodilator metabolites, released from any active tissue. Whatever they are they constitute a mechanism by which tissues can claim more blood flow whenever needed.

It is evident that the previously mentioned vasodilator fibers can, and do sometimes, contribute to increase the blood flow to activated tissues, but they are in no way indispensable; the local chemical factors are the important ones. Exactly what these factors are is still not clear, though soon 100 years of intense efforts will have been made to solve this problem. The reason why no one as yet identified *the* factor, responsible for the local dilatation, may simply be that there is not one but many contributory factors. If so, elimination of one factor does not change the dilatation much, though it normally is of importance. We do know that several factors, normally released, have a vasodilator influence. First the basic shifts in internal environment, such as decrease in oxygen tension, increase of carbon dioxide tension, or lowering of pH, should be mentioned; they all tend to relax vascular smooth muscle. It is reasonable to believe that they do so by a direct influence on the muscle cells and not only via a release of other dilator agents. This does not exclude that such factors cooperate. Of known tissue metabolites, carbon dioxide, lactic acid, and adenosine compounds appear to be the only ones that can be expected to cause any significant vasodilatation. It should further be remembered that on tissue activation a slight increase in extracellular potassium concentration takes place and such a chemical shift also has a vasodilator influence. The adenosine compounds, being released at the very start of cell excitation, have a profound vasodilator action, but no one knows whether they really diffuse out of the cells in significant amounts, which is the prerequisite to reach the blood vessels. Once again it has been suggested that also quite specific vasodilator agents should be produced. Thus histamine and acetylcholine have here been discussed, but there is nothing at present to indicate that they should play any significant role in this respect. The only known factor that can be called a specific vasodilator agent is the previously mentioned bradykinin, released from activated gland cells. It appears to be released only from this type of tissue, however, and may here play the role to cover the double demand of blood flow, both for metabolism and for production of secretion. On the whole, it may seem surprising that we know so little about this fundamental question, but the fact is that it is extremely difficult to attack experimentally.

The question now arises: is the blood flow per se regulated? In a way it is, in a way it is not, it depends very much on what is meant exactly by the word "regulated." The tissues have their local mechanisms capable of increasing their blood supply in a good balance to what is their actual nutritional demand, but the organism *as a whole* cannot always allow any tissue to make free use of its "blood flow reserve." There is an upper limit of cardiac output and often stress situations have seriously restricted the pump capacity. Under such circumstances the most important and most sensitive tissues, the heart and the brain, must be supplied first. Therefore, superimposed upon the local dilator control we have the restricting influence of the vasoconstrictor fiber system, so organized in its distribution, discharge, etc. that it has the power to give the biggest rations of the decreasing cardiac output to those tissues that need it most. It does so by maintaining as long as possible, a suitable perfusion pressure to these areas, which, due to the characteristics of constrictor fiber distribution are not exposed to flow restriction. Therefore, in discussing regulation of blood flow, one has to judge the steady balance between the local demands and the superimposed, restricting system, acting as the maintainer of a suitable perfusion pressure for the most important tissues. No doubt, there are here many important things that we still know too little about, and it cannot be said that we at present know exactly how the blood to the tissues is controlled or regulated.

Summario in Interlingua

Il es generalmente recognoscite que alterationes del tono in le musculatura lisie del vasos es exquisitemente importante in determinar alterationes del fluxo de sanguine. Es etiam generalmente acceptate como un facto que inter le factores que influe super le tono

vasomuscular le factor nervose es le plus importante, sed il remane un question controverse quante e qual mechanismos specific es interessate in iste relationes.

Le presente articulo summarisa le conception classic del influentias que determina le tono vascular, confrontante lo con le opiniones currente con attention special prestate al relation inter le mechanismo nervose e altere significative mechanismos. Es discutite separatemente le factores excitatori, que es primarimente nervose, e le factores inhibitori, inter le quales effectos hormonal pare esser de plus grande importantia.

In conclusion le autor subleva le question de si le studio del factores que determina le tono vasomuscular permitte le conclusion que il se tracta hic de un systema regulatori (per contrasto con mechanismos simplemente determinatori). Le autor summarisa su analyse de iste question in le assertion que multe importante factos in iste campo de investigation remane inadequatemente clar, de maniera que il non es possibile a iste tempore dicer con precision como le provision de sanguine al tissus es determinate o regulate.

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CLINICAL PROGRESS

Pharmacologic and Chemical Tests as an Aid in the Diagnosis of Pheochromocytoma

By GRACE M. ROTH, PH.D., EUNICE V. FLOCK, PH.D., WALTER F. KVALE, M.D., JOHN M. WAUGH, M.D., AND JAUNEITA OGG

ONE of the known causes of hypertension is a pheochromocytoma, a tumor of the medullary portion of the adrenal gland that secretes epinephrine or norepinephrine in varied but increased amounts. It produces either sustained hypertension or paroxysmal hypertension frequently associated with bizarre symptoms during the attack, together with hypermetabolism and high blood sugar in some instances. Although pheochromocytoma is rare, it is being diagnosed oftener and will become increasingly important with safe surgical removal. Thus the correct preoperative diagnosis becomes imperative and can sometimes be lifesaving.

An estimated 90 per cent of pheochromocytomas arise from the adrenal medulla, but they may occur wherever chromaffin tissue is found along the sympathetic nervous system. Of pheochromocytomas found outside the adrenal glands, 6 have been found in the thorax, 4 in the urinary bladder, and 18 in the organ of Zuckerkandl. Multiple tumors may occur in both adrenal glands or along the aorta in the sympathetic chain; some of the latter tumors may be metastatic, as approximately 10 per cent of pheochromocytomas are malignant. Familial pheochromocytoma has been reported.

From the Mayo Clinic and the Mayo Foundation, Rochester, Minn.

Read at the Thirty-first Scientific Sessions of the American Heart Association, San Francisco, Calif., October 24, 1958.

When the patient has paroxysmal hypertension, the attacks consist of a sudden rapid rise of the blood pressure with tachycardia, great anxiety, severe headache, pallor (particularly of the face), numbness, tingling and coldness of the hands and feet, sometimes nausea and vomiting, pain in the epigastrium extending into the precordial region and, lastly, excessive sweating. In any one case, one or more of these symptoms may be lacking. Occasionally a physician may be fortunate enough to observe a patient in one of these attacks, but often only the history of the attacks is available and it may be inconclusive.

For the patient with sustained hypertension the physician may not have even a history of attacks to point the way to a correct diagnosis. Today, however, pharmacologic and chemical tests are helpful in screening a relatively large number of patients for pheochromocytoma and are a definite aid to a correct diagnosis.

Pharmacologic Tests

Fifteen years[1] have elapsed since we first introduced the histamine test for pheochromocytoma. Although we have used all the drugs[2-5] suggested for testing as they appeared, we now use histamine for the patients with paroxysmal hypertension, because it stimulates discharge of pressor substances from the tumor and produces attacks similar to those occurring spontaneously, and we now use phentolamine hydrochloride (Regitine)[6, 7] for the patient with sustained hypertension,

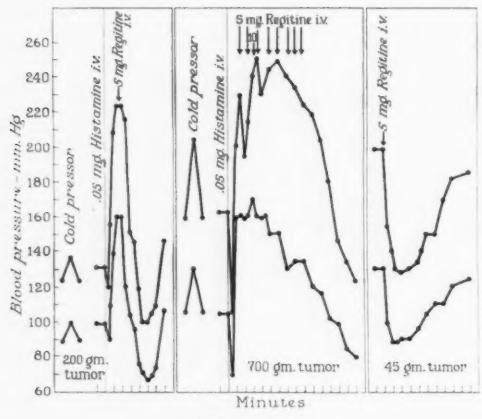

Figure 1

Changes in blood pressure during the cold pressor, histamine, and Regitine tests. Left and center. *Patient had paroxysmal hypertension.* Right. *Patient had sustained hypertension.*

because it decreases blood pressure by blocking the pressor effect of epinephrine or norepinephrine in the blood if a pheochromocytoma is present.

To carry out these tests correctly, certain prerequisites are necessary: The first is a reliable basal blood pressure. With the patient lying comfortably, the blood pressure is observed for at least half an hour to obtain the correct basal value. This is highly important in the interpretation of the results after use of histamine or Regitine.

Cold Pressor Test, a Prerequisite

Before either the histamine or the Regitine test, the cold pressor test[8] is performed, except when the diastolic blood pressure is more than 150 mm. Hg. The cold pressor test is done by immersing one of the patient's hands well above the wrist in a pail 8 inches high in water of 4 C. for 1 minute and measuring the blood pressure on the opposite arm at 15, 30, and 60 seconds during the immersion. The highest blood pressure during this painful stimulus indicates the lability of the blood pressure. If a patient has a history of blood pressure of 230/160 at one time and of 130/90 at another, the carefully determined basal blood pressure may be 130/90 and it may rise to 230/160 during the cold pressor test. Such a patient has very labile blood pressure and probably no pheochromocytoma, but if the rise in blood pressure during the cold pressor test is much less than to 230/160, pheochromocytoma may be suspected. The average increase in the blood pressure during the cold pressor test in our cases of proved pheochromocytoma was 40/29, with a range from 12 to 68 mm. systolic and from 10 to 78 mm. diastolic.

The cold pressor test is an integral part of the histamine test, since the response of the blood pressure to cold is used as the measuring stick for the response of the blood pressure 2 minutes after the intravenous injection of histamine. In our experience a cold pressor test has never produced an attack in a patient with pheochromocytoma because there is a sudden rapid rise in the blood pressure during the cold pressor test, whereas it is the sudden rapid fall in blood pressure that stimulates the secretion of the tumor. Furthermore, knowledge of the lability of the blood pressure is important on the third and fourth days after operation if a pheochromocytoma is removed, when pain may produce an elevation in blood pressure.

Histamine Test Followed by Regitine Test for Paroxysmal Hypertension

After the cold pressor test, when the blood pressure has returned to basal levels in the patient who has paroxysmal hypertension and a basal blood pressure of less than 170/110, 0.05 mg. of histamine base in 0.5 ml. of normal saline solution is injected intravenously with a tuberculin syringe. The needle is left in the vein, the empty syringe is removed immediately, and a syringe containing 5 mg. of Regitine is attached for immediate administration if the blood pressure becomes alarmingly high.[9]

Blood pressure is determined every 30 seconds for the next 2 minutes (fig. 1). It always decreases 30 seconds after the injection of histamine, or the histamine has not entered the vein. Immediately thereafter the blood pressure increases rapidly and usually is ma-

ium in 2 minutes. If a pheochromocytoma is present, the characteristic clinical signs and symptoms of a severe attack appear concomitantly with the increase in blood pressure to well above the cold pressor level. Regitine hydrochloride, 5 mg., is injected 2 minutes after the histamine or before, if the rise in blood pressure makes it necessary.

Within 1 minute after the injection of Regitine the clinical signs and symptoms of a severe attack will disappear and the blood pressure will decrease if a pheochromocytoma is present. Thus, histamine can be given safely, and 2 positive reactions will be noted. Occasionally, when a large or active tumor is present, after the first fall and cessation of symptoms on administration of Regitine, the blood pressure and symptoms may reappear and more Regitine may be needed. Recently, in a patient with a 700-Gm. tumor 50 mg. of Regitine were necessary to reduce the blood pressure to basal levels in 10 minutes (fig. 1). This knowledge was important in the care of the patient during operation, as 50 mg. of Regitine were necessary to keep the blood pressure at a safe level during operation.

If the patient has only mild essential hypertension, the blood pressure will not rise as high after the injection of histamine (that is, will rise little if any above the height during the cold pressor test), and the headache will not disappear nor will the blood pressure fall rapidly after the injection of Regitine. If Regitine is given alone to a patient with labile hypertension between paroxysms, the blood pressure may not change even in the presence of a tumor because the concentration of pressor amines is not elevated in the blood between attacks. Therefore, false-negative results will be obtained.

Regitine Test for Sustained Hypertension

For the patient with sustained hypertension (a basal blood pressure of more than 170/110), 5 mg. of Regitine hydrochloride are administered intravenously, not intramuscularly. If a pheochromocytoma is present, the blood pressure should decrease at least 35/25 in the first 3 or 4 minutes after injection and then should return to the previous basal level in 10 to 15 minutes (fig. 1). Regitine will cause a decrease in the blood pressure of some particularly apprehensive patients during the first 1 or 2 minutes after injection in the absence of a pheochromocytoma, but in the next minute the blood pressure returns toward the basal level and the test is considered as being negative for such tumors.

Why Are So Many False Results Reported from the Pharmacologic Tests?

The first difficulty comes from not obtaining a reliable basal blood pressure. The second may be failure to observe the blood pressure quickly enough as the timing is very precise.

The third may be a difference in the blood pressure of the 2 arms.[10] To eliminate this third source of error, the blood pressure is measured routinely in both arms in our laboratory; if any disparity is found, determinations are made simultaneously in both arms during pharmacologic tests. In 5 of our 66 patients with proved pheochromocytoma the simultaneous determination of blood pressures in both arms was necessary during the Regitine tests.

A fourth difficulty concerns previous medication.[11] Before any of these tests, administration of any sedative or narcotic should be prohibited for at least 48 hours and possible self-medication should be checked, or the pharmacologic tests may yield *false-positive results.* In patients with paroxysmal hypertension, sedatives inhibit the rise of blood pressure during the cold pressor test. As a result, the increase of blood pressure during the histamine test may be greater than that during the cold pressor test, and thus may suggest the presence of a tumor that does not exist.

In patients with sustained hypertension, sedatives and tranquilizers may cause a fall in blood pressure typical of that produced by pheochromocytomas following the intravenous administration of Regitine (fig. 2) and this would be a *false-positive result.*

On the other hand, various antihypertensive drugs may produce *false-negative results.* Since most of these drugs act longer than sedatives, the difficulties are even greater (fig. 2).

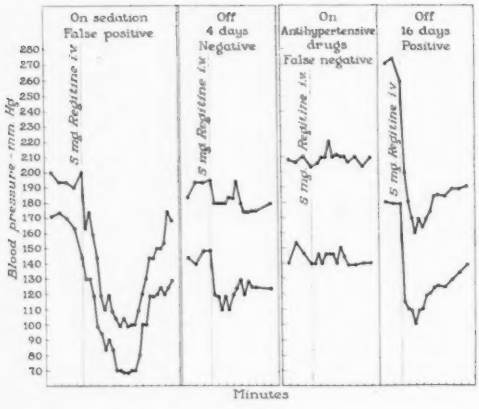

Figure 2

False-positive and false-negative results of tests with Regitine. (Reproduced with permission from the publishers and the authors. Roth, G. M., and Kvale, W. F.: Evaluation of pharmacologic tests as an aid in diagnosis of pheochromocytoma. With report of a case of pheochromocytoma and tuberculosis. Dis. Chest 32:292-302; 304, 1957.)

Regitine tests on 3 of our patients were negative while they were receiving antihypertensive drugs, but were positive after use of these drugs was stopped for 8 to 10 days. Tumors were subsequently found at operation.

The difficulties due to use of vasoconstricting drugs will be described with the pressor amines.

One patient had a good history of attacks for pheochromocytoma when lying on his left side, but our first pharmacologic tests on him gave negative results because he was taking various antihypertensive drugs. When he returned 6 months later, he had not been taking any drugs. During this examination basilar artery thrombosis and myocardial infarction occurred (fig. 3). Later in the hospital turning on his left side raised the blood pressure and produced severe headache. While he was lying on his back, his blood pressure was 130/90; when he turned onto his left side, it rose to 280/190. Blood drawn at the height of the blood pressure (fig. 3) contained 30.7 μg. of pressor amines per liter of plasma. When 5 mg. of Regitine were given, the blood pressure fell rapidly but rose again. This reaction

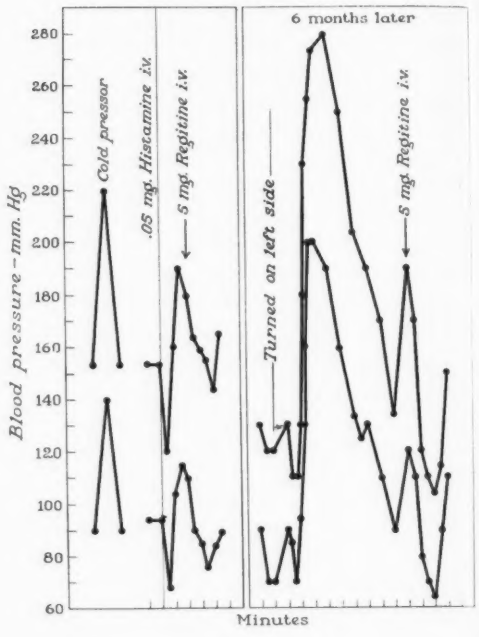

Figure 3

Left. *Cold pressor test and negative response to histamine and Regitine during administration of various drugs.* Right. *Six months later when the patient had had no drugs. After the patient turned on the left side the blood pressure rose from 130/90 to 280/190 and the classic severe headache, pallor, and excessive sweating occurred. On return to the supine position, blood pressure fell rapidly. An 80-Gm. tumor was removed later.*

was positive for pheochromocytoma. A tumor weighing 80 Gm. was successfully removed.

The ideal time to do the pharmacologic tests is before any antihypertensive drugs are given. If this is impossible, administration of antihypertensive drugs should be discontinued for 8 to 10 days.

Observations on Blood Pressure

In our series of patients with pheochromocytoma, the average basal blood pressure of those with normal blood pressure between episodes was 117/78, that of the patients with labile blood pressure was 151/105, and that of the patients with sustained hypertension was 215/146. The increases in blood pressure on the cold pressor test were the same for pa-

tients with normal blood pressure between attacks and for patients with labile blood pressure. Although the rise with histamine was slightly greater in the patients with normal blood pressure (108/59) than in those with labile blood pressure (75/46), the basal level was considerably higher for patients with labile blood pressure. Although we suggest that a fall in blood pressure of 35/25 may be considered as being a positive result of the Regitine test, the average fall on testing in our 19 patients who were later proved to have pheochromocytoma was 78/53.

Tests for Pressor Amines

Turning to the chemical tests on the blood, it seems obvious that if increased amounts of epinephrine and norepinephrine could be found in the blood at the height of the paroxysmal attack or in the blood of the patient with sustained hypertension, the accurate diagnosis of pheochromocytoma would be relatively simple. In 1953 fluorometric methods were developed for the determination of pressor amines in the plasma by Lund,[12] Natelson and associates,[13] and by Weil-Malherbe and Bone.[14, 15] The last method was modified by Manger and associates[16] for the separation of epinephrine and norepinephrine. For a time we used the Manger modification, but recently we have been determining only the total epinephrine-like substances by the method of Weil-Malherbe and Bone as an aid to diagnosis.

In our series of 490 patients without pheochromocytoma on whom 572 determinations of pressor amines (epinephrine-like substances in the plasma) were made, the mean values were 2.5 μg. per liter of plasma, with a range of 0.75 to 5.5 μg. The concentration of pressor amines in 36 patients with pheochromocytoma ranged from 6.9 to 360 μg. per liter of plasma.

Pitfalls

It has become evident on use, however, that the chemical tests for pheochromocytoma present pitfalls, just as do the pharmacologic tests. Blood collected when the tumor is not secreting will contain small or normal amounts of epinephrine and norepinephrine. However, if the blood is obtained before and at the maximal increase of blood pressure during a histamine test (fig. 4), the pressor amines are greatly increased in a patient with pheochromocytoma. Therefore, normal blood pressure and normal plasma pressor amines before histamine may be only a false-negative result.

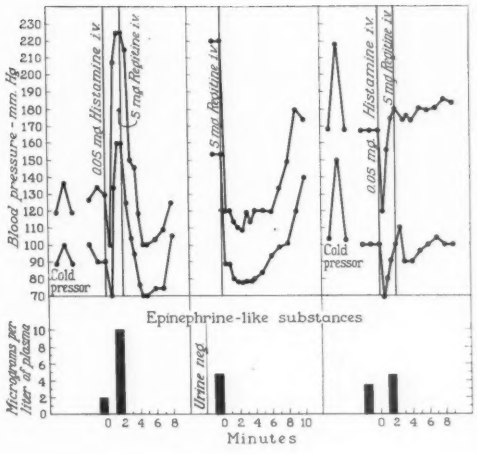

Figure 4

Left. *Normal blood pressure and normal pressor amines before positive histamine test.* Center. *Catecholamines negative elsewhere. With positive results of the Regitine test, pressor amines in blood were increased at the height of the blood pressure. An 80-Gm. tumor was removed later.* Right. *Negative results of histamine and Regitine tests with increased level of pressor amines. Exploration showed a lymphoblastoma, but no pheochromocytoma.*

In contrast, in certain conditions such as azotemia, jaundice, and lymphoblastoma, fluorescent substances other than pressor amines are present in the blood and appear with the technic used as increased amounts of pressor amines. In 1 patient with lymphoblastoma, results of the pharmacologic tests were negative for pheochromocytoma but the clinical findings and an elevation of pressor amines indicated a pheochromocytoma. An adrenal tumor was not found on exploration or later at necropsy (fig. 4).

Chlorpromazine (Thorazine) may increase fluorescent substances other than pressor amines in the blood; 1 patient with a high

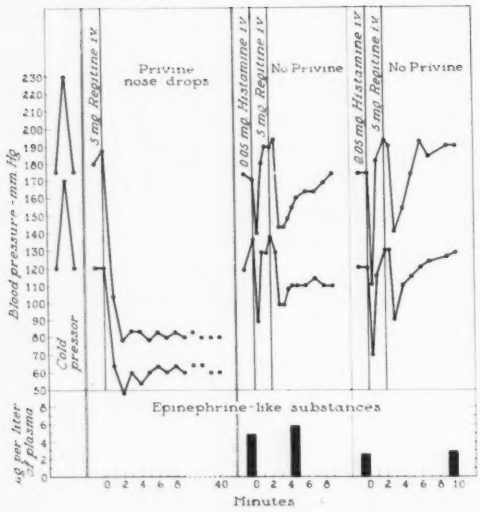

Figure 5

Left. *Note the elevation of blood pressure during the cold pressor test and rapid prolonged fall in blood pressure after administration of Regitine.* Center. *After the nose drops were discontinued, the fall in blood pressure following the injection of Regitine was much less.* Right. *Before the exploratory operation was carried out another test showed that the levels of pressor amines in blood had returned to normal. No tumor was found at operation.*

level of substances presumed to be pressor amines died and an adrenal tumor was not found. Tetracycline also causes the appearance of fluorescent substances other than pressor amines. A normal volunteer took 3 doses of tetracycline of 250 mg. each by mouth 6 hours apart. After the third dose, presumably the level of pressor amines had increased from 1.9 to 6.9 μg. per liter of plasma. Extremely high levels (7.4 to 40 μg. per 100 ml. of plasma) of fluorescent substances were found in 4 patients receiving this antibiotic. After administration of the drug was stopped for 4 to 6 days, these values decreased. One of these patients was explored and no adrenal tumor was found. It was significant that results of the Regitine tests were negative in these patients. Other drugs also may have such fluorescent properties.

Two further difficulties have arisen. First, there is evidence that the concentration of pressor amines may be depressed in a patient with pheochromocytoma during the early administration of antihypertensive drugs. However, the growth of the tumor is not inhibited and, as it grows, the increase in levels of pressor amines may be re-established and blood pressure may increase even though use of the antihypertensive drug is continued.

An annoying side reaction to Rauwolfia is nasal stuffiness.[17] Many vasoconstrictors are used locally for this difficulty and also for vasomotor rhinitis and asthma. These substances produce high levels of pressor amines in the blood and increased amounts of catecholamines in the urine. Such increases have been interpreted as due to pheochromocytoma. These vasoconstrictive drugs also produce a profound fall in blood pressure for as long as 40 minutes after the intravenous injection of Regitine (fig. 5). In contrast, in the presence of a pheochromocytoma the fall in blood pressure may be as great, but the pressure begins to increase in 3 to 4 minutes and returns to the basal level in 10 to 15 minutes. The prolonged lowering of blood pressure from the vasoconstrictor drugs after injection of Regitine alerts the physician to the possibility that something other than a pheochromocytoma is producing the response. We have never administered any vasoconstrictor drug during this prolongation of lowered blood pressure. Six other patients who came with a history of positive pharmacologic tests and elevated pressor amines were using nasal vasoconstrictor drugs. After use of the drugs was stopped the tests were negative.

Forty-five additional determinations for pressor amines on patients who had been receiving vasconstrictor substances for nasal stuffiness or asthma, or tetracycline, gave an average of 8.2 μg. per liter of plasma, with a range of 4 to 40 μg.

An Unusual Circumstance Giving False-Positive Reactions

An interesting diagnostic problem was presented by a 21-year-old paraplegic patient with normal levels of blood pressure who periodically had severe headaches, excessive sweating, and a sig-

fi ant elevation in blood pressure. A pheochromo-
c oma in the urinary bladder was suspected. The
c d pressor test showed a slight hyperreaction.
T e initial blood pressure was low, but during the
n xt 40 minutes it rose to 210/132, with sweating,
s ne shaking, and a severe headache (fig. 6). Blood
d wn at this time showed an elevated level of
p essor amines. Administration of 5 mg. of Regi-
t e produced a positive reaction for pheochromocy-
t na. At the end of 10 minutes when the blood
p essure was lower, the sweating ceased.

Because of previous experience with paraplegic
p tients, the bladder was emptied. Blood pressure
r turned to normal, and a histamine test carried
o t 30 minutes later gave negative results but the
l el of pressor amines in the plasma was still
el vated at that time.

The next day, after the bladder was emptied, a other histamine test gave negative results, and concentrations of pressor amines and catecholamines were found to be normal also. By filling the bladder with air in increments of 20 to 30 ml., the blood pressure could be elevated to 210/132, with sweating and headache. The symptoms were due to the mass reflex produced by filling of the bladder in a paraplegic patient.

Correlation of Results of Pharmacologic Tests and Tests for Pressor Amines with Failure to Find Pheochromocytoma at Operation

In our laboratory 13,834 pharmacologic tests were carried out on 12,713 patients. Tumors were diagnosed and removed at operation from 66 patients. No tumors were found in an additional 16 patients who underwent exploratory operation because of one positive test, or for clinical reasons, or who had a positive test but died before any operation. Eight of these 16 patients reacted positively to 1 Regitine test, but of these 8, 6 subsequently had a negative result from one or more Regitine tests before operation. Two of the 8 had high values for pressor amines (positive finding) because of epinephrine in nose drops. Of the other 8 patients, 2 had positive results of the test for pressor amines but the results of the Regitine tests were negative; 3 had a positive result from one histamine test but negative results were obtained on other histamine tests before operation. The false-positive results to the histamine tests were due in 1 patient to Regitine taken by mouth for 2 months, in 1 to sedation for headache, and in 1 to a severe migraine headache.

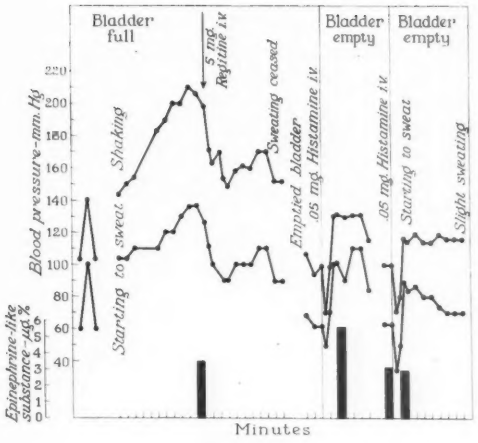

Figure 6

Abnormal pressor responses in paraplegic patient with full bladder.

The tests on the other 3 patients gave negative results, but exploratory operations were carried out for clinical reasons.

In contrast, recently 1 patient who had taken various drugs and frequently had some attacks with hyperventilation before he arrived for the pharmacologic and chemical tests had negative results of histamine and Regitine tests and normal levels of catecholamines in both the plasma and urine on 5 different days during a period of 21 months. We did find, however, that 2 minutes after exercise and hyperventilation (that is, 2 minutes after bending and standing for 2 minutes and hyperventilating for 5 minutes) the blood pressure rose from 150/116 to 194/120. Following this procedure the pressor amines rose from 3.0 to 9.8 µg. per liter of plasma when the blood pressure was 194/120. Urine collected half an hour later contained 40 µg. per 100 ml. A 32-Gm. tumor was removed.

Catecholamines

Turning to urinary catecholamines, in 1950 Engel and von Euler[18, 19] indicated that the biologic assays of catecholamines reported by Holtz and associates[20] and later by Helmer[21] and themselves could be used in the diagnosis of pheochromocytoma. In 1951 Goldenberg and Rapport [22, 23] modified the von Euler test

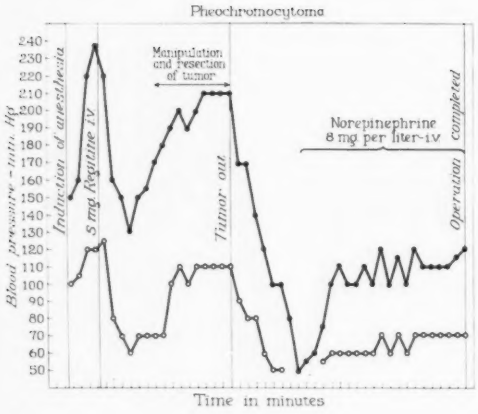

Figure 7

Variations in blood pressure during operation for removal of a pheochromocytoma.

and made it a chemical method for determination of catecholamines in the urine, which they consider the best test. Numerous methods are now available for determining catecholamines.

At present we are using the von Euler and Floding methods[24] for determining catecholamines in the urine as modified by Sobel and Henry.[25] In a series of 290 specimens of urine from 287 patients without pheochromocytoma the average concentration of total catecholamines was 9.4 μg. per 100 ml. The average 24-hour output was 113 μg. In 4 of our patients with proved pheochromocytoma, the catecholamines in the urine 30 minutes after injection of histamine ranged from 42 to 2,649 μg. per 100 ml. After removal of the tumor, the patient who excreted 2,649 μg. per 100 ml. (48.0 μg. per mg. of creatinine) excreted 6 μg. per 100 ml. (6.6 μg. per mg. of creatinine) 30 minutes after the histamine test.

The fact still remains, however, that a pheochromocytoma may not secrete continuously, and normal levels of catecholamines may be obtained. On the other hand there are reports[26] of excretion of normal levels even when attacks have occurred spontaneously or have been induced with histamine. Also, patients using vasoconstrictor substances have shown increased levels of catecholamines. With the finding of norepinephrine in bananas, more excretion of norepinephrine may be explained on this basis.

Surgical Aspects and Precautions

Various hazardous conditions may occur before, during, and after operation, and certain precautions should be taken. Before operation we have found that many active tumors are stimulated by the fall in blood pressure after the hypodermic administration of as little as 50 mg. of meperidine hydrochloride (Demerol) and 200 grains of phenobarbital (Luminal). Alarmingly high blood pressures may ensue that can be counteracted by an intravenous injection of Regitine, which should be at hand. With the induction of anesthesia the blood pressure again may rise to excessive heights (fig. 7). It has been our custom to take the blood pressures at minute intervals during the operation and to give Regitine when needed in order that the level of blood pressure may be safe for the surgical procedures being performed. Whenever the tumor is stimulated by manipulation, the blood pressure may rise and Regitine may or may not be necessary. When the tumor is removed, a sudden fall in the blood pressure takes place. It is wise to wait a few minutes before proceeding with the operation to determine whether the blood pressure rises again. The fall after removal of the tumor may stimulate secretion from another tumor, if one is present, that will increase the blood pressure. We have looked for and found additional tumors because of such observations. If the fall in blood pressure is maintained for several minutes, norepinephrine (levarterenol, Levophed) is administered. The amount of norepinephrine required and the length of time that this substitution therapy is necessary varies from patient to patient. Little or no prediction can be made. It may be necessary to give norepinephrine at intervals from 6 to 72 hours, and in a couple of instances none was needed.

Another precaution can be suggested for general surgery. If a hypertensive reaction occurs during any operative procedure, the presence of a pheochromocytoma might be suspected. Regitine could be administered to

onfirm the diagnosis of pheochromocytoma, nd the use of this drug could protect the atient until the pheochromocytoma is re-oved. Bartels and Cattell[27] have reported 4 atalities during lumbar sympathectomy, re-air of an incisional hernia, a thoracic opera-ion, and a gastrectomy due to unsuspected heochromocytoma. In our experience a hy-ertensive crisis occurred in the course of an peration for removal of the gallbladder; the drenal gland was explored, a pheochromocy-oma found and removed, and then the gall-bladder was removed at the same operation.

Discussion

The correct diagnosis of pheochromocytoma is still difficult, and the surgical excision is a hazardous procedure unless the blood pressure is controlled during and after the operation. Thus pheochromocytoma is a challenge to the clinician, to the physiologist, to the biochemist, and to the surgeon.

The greatest obstacle to the successful diagnosis of pheochromocytoma from pharmacologic and chemical tests is previous medication. For determinations of the pressor amines in the plasma and urinary catecholamines of patients with paroxysmal hypertension, stimulation of the adrenal tumor is often necessary for the increased excretion indicative of pheochromocytoma. No one test is infallible, and knowledge concerning the drugs used or the manner in which the blood and urine are collected, or both, is necessary to avoid a false-positive or false-negative result from the test.

The Regitine test following the histamine test is a good index of the amount of Regitine necessary to control the blood pressure during operation and gives some indication of the amount of norepinephrine (Levophed) to be prepared for use following removal of the tumor.

The most frequently asked questions are (1) how many patients are screened to find the large number of adrenal tumors, and (2) how many deaths have occurred during operation? To July 1, 1959, we have carried out 13,834 pharmacologic tests on 12,713 patients, 994 tests for pressor amines on 526 patients, and 305 tests for catecholamines on 293 patients. Seventy-six pheochromocytomas have been diagnosed and successfully removed from 66 patients at the Mayo Clinic. No untoward effects or deaths have occurred in our cases during the pharmacologic tests, during operation, or immediately after operation.

Summary

Today pharmacologic and chemical tests are helpful in screening a relatively large number of patients for pheochromocytoma and are a definite aid to a correct diagnosis. No one test is infallible, and knowledge concerning the drugs used or the manner in which the blood and urine are collected, or both, is necessary to avoid a false-positive or false-negative result from the tests.

Summario in Interlingua

In nostre dies, tests pharmacologic e chimic es de adjuta in examinar relativemente grande numeros de patientes pro deteger pheochromocytoma. Illos es definitemente utile in establir le correcte diagnose. Nulle test individual es infallibile. Un detaliate cognoscentia del drogas usate e del maniera in que le sanguine e le urina es colligite debe esser disponibile si on vole evitar resultatos false-positive o false-negative.

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Concluding Articles of Symposium on Regulation of the Cardiovascular System in Health and Disease
To Appear in June 1960 Issue

Regulation of Blood Volume. ..... *E. B. Reeve*
Regulation of Water and Electrolytes. ..... *J. Russell Elkinton*

ABSTRACTS

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CORONARY ARTERY DISEASE

Currarino, G., Silverman, F. N., and Landing, B. H.: Abnormal Congenital Fistulous Communications of the Coronary Arteries. Am. J. Roentgenol. **82**: 392 (Sept.), 1959.

Three instances of congenital fistulous communications between a coronary artery and a cardiac chamber are reported. In 1 patient, an 11-year-old child, a fistulous communication between an enlarged right coronary artery and the right ventricle was demonstrated by retrograde aortography. The second case was that of an infant who, at autopsy, showed an enlarged supernumerary aortic coronary artery communicating with the right atrium. An autopsy on the third patient, an infant, revealed a congenital atresia of the pulmonary artery orifice with intact ventricular septum and an enlarged right coronary artery that drained into the right ventricle. Congenital communication anomalies of the coronary arteries consisted of a fistulous connection between a main coronary artery or 1 of its branches and structures outside of the heart, the main pulmonary artery, another coronary artery, a coronary vein or the coronary sinus and, a cardiac chamber. The fistulous tract and the vessel from which it arose were often enlarged and tortuous and at times aneurysmal. The nature of these malformations was not certain but microscopic communications normally connect the coronary arteries with all the structures mentioned. It is possible that the fistulous form resulted from dilation of normal channels rather than a truely developmental anomaly. The literature is reviewed and clinical remarks are also included.

KRAUSE

Di Cantogno, V. L., Chiche, P., and Baillet, J.: Subendocardial Ischemia or Generalized Coronary Insufficiency. Anatomapathologic Considerations. Arch. Inst. cardiol. México **29**: 65 (Jan.-Feb.), 1959.

A critical analysis of the similarities and differences between subendocardial anoxemic necrosis and myocardial infarction was made. In spite of their variability, subendocardial lesions always showed a marked contrast between the intensity of the interfibrillary edematous reaction and the relative discreteness of the myocardial necrotic changes. Clinically the word "infarction" is relegated to a limited significance, describing the massive destruction of a well-defined zone in the ventricular wall, almost always secondary to acute occlusion of a coronary artery. On the other hand, subendocardial necrosis refers to patchy lesions irregularly disseminated in the deep muscular layers of the left ventricle. The lesions do not have a precise limitation and, furthermore, they are reversible when the fibers are slightly injured. Subendocardial ischemia secondary to generalized coronary insufficiency either cardiac or extracardiac, anatomic or functional, may occur in some conditions that are still placed in the border between physiologic and pathologic (tachycardia, transient imbalance of coronary flow, etc.) or may be the expression of a definitely pathologic condition (coronary occlusion, thrombosis, ostial stenosis, shock, etc.). The prognosis depends upon the cause of the coronary deficit rather than on the myocardial alterations.

BRACHFELD

Garamella, J. A., Andersen, J. G., Oropez, R., Velosa, A., Song, K. T., Hay, L. J., and Naidu, R.: Antifibrillatory Agents and Surgery Tested by Closed Chest Coronary Occlusion in the Pig. Surgery **45**: 292 (Feb.), 1959.

The authors studied 145 apparently healthy stockyard pigs (12 to 30 Kg. weight range) with a reliable method of acute closed-chest coronary artery occlusion. In the control group of 45 pigs, fatal ventricular fibrillation occurred 1½ to 5 minutes following the occlusion in two thirds of the animals. The longest survival time in this group was 27½ minutes. Pretreatment by antifibrillatory agents gave the following total acute and chronic survival percentages: Roche 2-5803 44.4 per cent (acute 5.5, chronic 38.9); Quinidine 36.6 per cent (acute 20, chronic 16.6); Pronestyl 6.6 per cent (acute 6.6). Pretreatment by various surgical procedures was also investigated. Acute internal mammary artery ligation afforded no protection. Internal mammary artery ligation with coronary artery occlusion performed 58 to 69 days afterwards resulted in 33.3 per cent survival. The Beck I operation followed by coronary artery occlusion 52 to 102 days later gave 27.7 per cent protection. It was concluded that the incidence of ventricular fibrillation in the pig following acute closed-chest occlusion of the left anterior descending coronary artery at its bifurcation could be reduced by several antifibrillatory agents and surgical procedures.

KITCHELL

Himbert, J., Lenègre, J., and Tintoret-Ferrer, S.: Anatomic and Electrocardiographic Study of 15 Lateral Myocardial Infarctions. Arch. mal. coeur **52**: 841 (Aug.), 1959.

Of 204 anatomic and clinical observations of myocardial infarction, pure lateral localization was found in 15 cases (7.3 per cent). Of the 2 patients showing no specific signs (only a left ventricular hypertrophy and "strain" pattern expected in hypertension), 1 had a very small subendocardial and 1 a small subepicardial infarction; in the former case wide and deep Q waves were at first present in leads I and V_6, but disappeared after 2 years. In 1 case S-T depression in V_5 to V_7 and in 2 S-T elevation or pointed inverted T waves in $V_{6\text{-}7}$ were the only suggestive signs. The remaining 10 cases showed deep and wide Q waves in leads V_7, $V_{6\text{-}7}$, or $V_{4\text{-}7}$; in 4 these signs were present also in leads I and aV_L, while in 5 they were present also in leads III and aV_F; extension toward the posterior wall was present in only 3 of these. This extension depends on the length of the left circumflex artery. The distribution of the electrocardiographic signs on the limb leads accordingly depends more on cardiac rotation than on the actual extension of the infarcted area. If the standard limb and precordial leads show only borderline changes, leads V_8 and V_9 or high precordial leads may prove more definite, especially if in V_1 R exceeds S. Comparison with previous tracings is also of great importance in questionable cases.

LEPESCHKIN

Jestädt, R., and Sandritter, W.: Experience with the TTC Reaction (Triphenyltetrazolium Chloride) in the Pathologic Diagnosis of Fresh Myocardial Infarction. Ztsch. Kreislaufforsch. 48: 802 (Sept.), 1959.

In 112 cases of fresh myocardial infarction, sections of ventricular muscle were incubated ½ hour at 37 C. in buffered TTC solution. Absence of the usual red coloring due to presence of dehydrogenases was usually found in a larger area than that corresponding to macroscopically visible myocardial necrosis. In 44 cases no myocardial necrosis was visible macroscopically but the TTC reaction showed absence of dehydrogenases, and in 11 of these no microscopic signs of necrosis could be found. In 4 cases of infarction occurring 1 to 3 hours before death both histologic appearance and TTC reaction were normal. On the other hand, the TTC reaction was absent in spots in 5 of 40 hearts without coronary sclerosis; in some of these, contact between the specimen and the solution may have been deficient at the bottom of the glass. In 14 hearts with fatty degeneration and in 1 with interstitial myocarditis, spotty absence of the reaction was found. In 28 rabbits the reaction was negative in the region of experimental myocardial infarction 3 to 4 hours after coronary ligation, but in these cases histologic changes were also found in this region. Absence of dehydrogenases, which escape from the heart muscle due to increased permeability as a result of anoxia, can accordingly be of great importance in the postmortem diagnosis of fresh myocardial infarction, if it occurs at least 3 or 4 hours before death.

LEPESCHKIN

Jolliffe, N., and Archer, M.: Statistical Associations between International Coronary Heart Disease Death Rates and Certain Environmental Factors. J. Chron. Dis. **9**: 636 (June), 1959.

A new approach to the study of the relationship between diet and coronary heart disease was used in analyzing international food and mortality statistics gathered in 20 countries by 2 United Nations agencies. Saturated fats were distinguished from polyunsaturated fats, and indepen-

ent variables were treated in pairs. Countries such as the United States, Australia, Canada, Finland, New Zealand, and the United Kingdom showed a high death rate in coronary heart disease in men aged 55 to 59 years and also with high caloric, high animal protein, and high fat intake, especially saturated types of fat. In Norway, Sweden, and Denmark, where the total fat intake is high but where the percentage of unsaturated fats is higher than in other Western countries, death rates from coronary artery disease were lower. In countries where there are many telephones, the proportion of saturated types of fat in the diet was higher! Whatever the explanation for the increase in saturated fat consumption, the authors believe that environmental factors play an important role in the differences of death rates from coronary heart disease among countries. The saturated food fats studied comprised land animals, eggs, cow's milk, lard, tallow, coconut, and cocoabean. The polyunsaturated fats included vegetable oils and marine mammals.

MAXWELL

Kahn, P. M., and Prozan, G. B.: Hyperuricemia—Relationship to Hypercholesteremia and Acute Myocardial Infarction. J.A.M.A. **170:** 1909 (Aug. 15), 1959.

Hyperuricemia appears to be a concomitant of myocardial infarction with practically the same degree of frequency as hypercholesteremia. Of 35 men studied, 79 per cent, showed serum uric acid levels above 6 mg. per 100 ml. It is recommended that in assessing proneness to coronary heart disease the serum uric acid be considered. It is also advisable to use probenecid in the presence of hyperuricemia as prophylaxis against coronary disease.

KITCHELL

Levinson, D. C., Shubin, H., Gunther, L., and Meehan, J. P.: Hemodynamic Findings in Heart Block with Slow Ventricular Rates. Am. J. Cardiol. **4:** 440 (Oct.), 1959.

Right heart catheterization was done in 6 elderly patients with ventricular rates below 40 per minute due to partial or complete heart block resulting from coronary artery disease. The right atrial, right ventricular, and pulmonary artery systolic pressures were slightly or moderately elevated while the mean brachial artery pressure was reduced. The vascular resistance both in the pulmonary and systemic circuits was elevated. Oxygen consumption and cardiac index were reduced although in most patients the stroke volume was slightly increased. A waves were transmitted both to the right ventricle and pulmonary artery, and these waves were gigantic when concurrent with a closed tricuspid valve. The injection of 0.15 mg. of isoproterenol into the pulmonary artery of 2 patients was followed by an increase in ventricular rate and cardiac output, a decrease in arteriovenous oxygen difference, a decrease in pulmonary and systemic vascular resistances; there was no change in oxygen consumption or stroke volume.

ROGERS

Prinzmetal, M., Kennamer, R., Merliss, R., Wada, T., and Bor, N.: Angina Pectoris. I. A Variant Form of Angina Pectoris, Preliminary Report. Am. J. Med. **27:** 375 (Sept.), 1959.

Thirty-two cases are described as a variant of angina pectoris lacking the 2 major characteristics of the classic form: relation to effort with relief by nitroglycerin and electrocardiographic changes during the pain consisting of S-T depression in standard limb leads. In this variant, pain is not precipitated by effort but occurs at rest or during ordinary activity during the day or night. During an attack, S-T segments are elevated with reciprocal depression in the standard leads. The pain of the variant is often cyclic and periodic, frequently described as waxing and waning. It is suggested that this syndrome results from temporary occlusion of a large diseased artery due to increased vessel tonus. In 3 autopsied cases a tiny but patent lumen was found in a large diseased coronary artery. The variant angina may end in progressive coronary artery disease, remission, or death. Therapy may include anticoagulants, long-acting nitrites, antiarrhythmic drugs. Nylidrin, a sympathetic vasodilator, offers promise.

KURLAND

Rowe, G. G., Maxwell, G. M., Castillo, C. A., Crumpton, C. W., Botham, R. J., and Young, W. P.: Evaluation of the Effect of Bilateral Internal-Mammary-Artery Ligation on Cardiac Output and Coronary Blood Flow. New England J. Med. **261:** 653 (Sept. 24), 1959.

In 5 patients with angina pectoris the cardiac output and the coronary blood flow were determined before and after bilateral internal mammary artery ligation. Neither the coronary blood flow nor the coronary-sinus oxygen content were consistently changed by the procedure. It is concluded that this study is in agreement with others, indicating that the beneficial effect of internal-mammary-artery ligation on angina pectoris is probably only a placebo effect.

SAGALL

Shipp, J. C., Sidel V. W., Donaldson, R. M. Jr., and Gray, S. J.: Serious Complications of Peptic Ulcer after Acute Myocardial Infarction. New England J. Med. **261**: 222 (July 30), 1959.

Attention is called to the fact that in certain patients an acute myocardial infarction may constitute an acute ulcerogenic stimulus. The histories of 6 patients who developed severe ulcer complications within 1 to 24 hours after documented acute myocardial infarction are reported. Two suffered massive gastrointestinal hemorrhage and 4 developed perforation of an ulcer. In 10 other patients with documented myocardial infarctions but with no history suggesting peptic ulcer disease, an abnormally increased urinary pepsinogen excretion was found in 4. The uropepsin excretion in these 4 patients gradually decreased to normal levels during the period of convalescence. A comparable increase was not found in 10 control patients with coronary artery disease without recent infarction. The adrenal response, as measured by urinary excretion of 17-ketosteroids and 17-hydroxycortocoids, in the patients with acute myocardial infarction did not differ from that of the control group. In view of the findings reported the authors recommend that in a patient with acute myocardial infarction together with past or present symptoms of peptic ulcer an ulcer regimen be instituted prophylactically and that anticoagulants be withheld.

SAGALL

ELECTROCARDIOGRAPHY, VECTORCARDIOGRAPHY, BALLISTOCARDIOGRAPHY, AND OTHER GRAPHIC TECHNICS

Burch, G. E., and DePasquale, N.: The Electrocardiogram and Ventricular Gradient in Atrial Septal Defect. Am. Heart J. **58**: 190 (Aug.), 1959.

The electrocardiograms in 100 instances of atrial septal defect proved by catheterization, surgery, or autopsy were reviewed and studied. The electrocardiographic picture was found to be fairly characteristic with prominence (large magnitude or duration) of the S waves in leads I, V_5, and V_6 and of R′ waves in leads V_1 and V_2. With septum defects there was, in addition, left axis deviation of the QRS or $\hat{A}_{QRS}$; this did not appear in any of the 90 patients with septum secundum defect. With septum secundum defects the axis was shifted toward the right, resulting in an S wave of considerable magnitude in leads II and III. In spite of right atrial enlargement the P wave was not particularly altered, except in lead V_1. Although the electrocardiograms in these patients showed patterns suggestive of right bundle-branch block it is pointed out that, in line with previous studies, the mechanism for this pattern is not that of an interference with conduction through the right bundle branch and that, therefore, the arbitrary electrocardiographic criteria of right bundle-branch block are unreliable. The results of this study further indicate the value of the electrocardiogram in differentiating hypertrophy of the crista supraventricularis from that of the entire right ventricle.

SAGALL

Clark, L. C., Jr., and Bargeron, L. M., Jr.: Left-to-Right Shunt Detection by an Intravascular Electrode with Hydrogen as an Indicator. Science **130**: 709 (Sept. 18), 1959.

A platinum black electrode produces sizable potentials, with respect to a reference electrode, when exposed to blood containing hydrogen. Potentials of up to 300 mv. are observed, and these are relatively simple to record, even in the presence of the potentials generated by the heart. Platinum black electrodes retained their sensitivity over several weeks even though allowed to become dry, repeatedly exposed to blood, cleaned, and sterilized by soaking in 70 per cent alcohol or by autoclaving. A silver chloride-coated silver plate brought in contact with the skin through a saline-soaked pad was the most convenient reference electrode. A child with known pulmonary stenosis and ventricular septal defect and a predominant left-to-right shunt was studied. The electrode-tipped catheter was first located in the right ventricle and then withdrawn into the inferior vena cava. An inspiration of hydrogen was made with the catheter at each location. A 2-second-delay fast-slope curve was obtained for the right ventricle demonstrating the existence of a left-to-right shunt. This was subsequently confirmed by blood oxygen analysis and cineangiography. The potentials developed by the platinum black-tipped catheters are not strictly quantitative, but the differences in timing rate of response and final potential are so great that there is little difficulty in distinguishing between left and right heart blood after inspiration of hydrogen. The authors at present are attempting to develop a multiple electrode catheter whereby hydrogen potential measurements may be made simultaneously at various points and where blood samples and pressure measurements can be taken at the same time.

LEVINSON

Conrad, L. L., and Cuddy, T. E.: Activation of the Free Wall of the Right Ventricle in Experimental Right Bundle Branch Block. Circulation Research 7: 173 (Mar.), 1959.

The activation of the free wall of the right ventricle was studied in dogs after right bundle-branch block was surgically induced. Analysis of multiple electrode recordings indicated that after right bundle-branch block excitation spread radially at the earliest activated endocardial point and tangentially at later activated points. Endocardial spread was slower after block, and conduction through a specialized syncytium was either absent or greatly altered. The authors indicate that the altered pattern of activation in right bundle-branch block precludes any estimation of right ventricular wall thickness by measurement of the time of onset of the intrinsic deflection.

PAUL

Hanson, H. E., Jönsson, G., and Karnell, J.: Catheterization and Selective Roentgenographic Demonstration of the Left Ventricle of the Heart. Acta Radiologica 52: 33 (July), 1959.

Catheterization of the left ventricle provides valuable information in the following conditions: ventricular septal defect with left-to-right shunt, common atrioventricular canal, aortic valvular stenosis, subaortic stenosis, aortic valvular incompetence, mitral insufficiency, combined disease of the mitral and aortic valves, and pathologic lesions in the ventricular walls such as tumors and fibroelastosis. The technic utilized was to insert a soft thin-walled catheter through an exposed radial artery. The artery was ligated after the examination. The method for passing the catheter through the aortic orifice is described in detail. The great advantage of this technic is that pressure measurement may be carried out without haste, and with the catheter in position there is time to give consideration to the question of where and how the contrast medium, isotopes, or dye should be injected to obtain the most information.

SHEPS

Heckmann, K., and Haubrich, R.: The Electrokymogram in Atrial Septum Defect. Fortschr. Röntgenstr. 91: 172 (Aug.), 1959.

In atrial septum defect the electrokymogram of the right atrium shows a deeper and more prolonged presystolic descent and a diminished diastolic descent (diastolic plateau). The left atrial curve also shows an accentuated presystolic descent but the diastolic descent is also accentuated. The pulmonary artery shows a more rapid outward movement, an earlier summit and a lower location of the incisura. These changes can be attributed to increased pulmonary flow. The aortic curve may show a lower incisura. The same characteristics can be found also in ventricular septum defects, but in this case the left ventricular curve shows a characteristic earlier onset of the systolic inward movement, which may occur during the isometric tension period. Development of pulmonary hypertension and shunt reversal causes the pulmonary curve to assume the characteristics of the aortic curve and vice versa, but the curve from the pulmonary artery branches in the right hilus retains the characteristic early summit.

LEPESCHKIN

Koechlin, R., Testoni, F., Narbonne, N. B., and de Bonis.: Vectocardiographic Study of the Symmetry of the Cardiac Electric Field in Normal and Pathologic Subjects. Arch. mal. coeur 52: 895, (Aug.), 1959.

In 10 persons with normal and 20 with abnormal electrocardiograms, vectorcardiograms registered with lead I as the transverse, V_F as the vertical, and V_2o as the sagittal components were compared with those obtained by replacing 1 or more of these components with V_6o-$V_{6R}o$, the bipolar lead $V_{6R}o$-V_6o-V_8o (left supraclavicular fossa), the bipolar lead V_8o-V_F-$V_{2P}o$ (projection of V_2o on the back) and the bipolar lead $V_{2P}o$-V_2o. The precordial positions and amplifications (o) were determined for each individual so that they gave the best approximation to the corresponding limb-lead component. This was accomplished by displaying them at right angles to each other until the resultant vector loop showed the best approximation to a straight line. A total of 6 to 26 vectorcardiograms was registered for each person. In most subjects the QRS vector loops registered by the different methods were nearly identical while the T loops showed minor discrepancies. The conclusion is made that in most cases the heart behaves as a single dipole once the apparent center of this dipole is determined.

LEPESCHKIN

Kubat, K., Dejdar, R., Widimsky, J., and Valach, A.: The Electrokymographic Picture of the Heart and Great Vessels in Chronic Cor Pulmonale. Cardiologia 35: 207, 1959.

In patients with chronic cor pulmonale and latent pulmonary hypertension electrokymographic investigations showed that filling of the pulmonary artery occurred after a longer than normal interval from the start of the QRS com-

plex in the electrocardiogram and later than in the aorta. The interval between filling of the 2 great vessels was often longer than in normal subjects. In the pathologic cases the tracing of the pulmonary artery attained its maximum only at the time of the second heart sound, and the incisura and the dicrotic wave were often absent. These signs were more marked the more severe the clinical condition. A normal electrokymogram did not exclude pulmonary hypertension with certainty, particularly if hypertension became evident only with exercise.

BRACHFELD

Porstmann, W., and Geibler, W. Retrograde Catheterization of the Left Ventricle from the Femoral and Right Carotid Arteries: Indications and Results of Two Complimentary Methods. Fortschr. Röntgenstr. 91: 151, (July), 1959.

Percutaneous catheterization of the left ventricle through the femoral artery is the least dangerous of the 2 methods. The degree and location of an aortic stenosis can be best demonstrated by continuous pressure measurement during withdrawal of the catheter, the degree of aortic regurgitation by injection of contrast medium into the aortic arch. In the presence of major stenosis the left ventricle can sometimes not be reached in this way, but injection of dye against the aortic valve can still show the stenosis due to the diluting jet effect. Catheterization through the exposed right common carotid artery was possible in 21 of 25 cases of severe aortic stenosis. Demonstration of dye regurgitation into the left atrium is diagnostic of mitral insufficiency only if sinus rhythm is present; if ventricular extrasystoles are elicited by the dye injection, it can occur with normal valves.

LEPESCHKIN

Pruche, A.: Importance of High Amplification in Electrocardiography. Morphologic Analysis of Tracings. Normal Subjects. Arch. mal. coeur 52: 400 (April), 1959.

In normal persons unipolar leads were taken at a sensitivity of 10.12 cm. per mv. from 5 radiologically controlled points of the precordial region. The characteristic configuration of these leads is described. Some of the patterns not previously described were a double summit of the U wave that became single and greatly elevated after exercise; a notch at the beginning of T, that migrated to the ascending branch of T when the heart rate became slower; and a U wave that became greatly elevated after long diastolic pauses in respiratory arrhythmia. The last observation favors the interpretation of the U wave as being caused by protodiastolic ventricular filling.

LEPESCHKIN

Sodi-Pallares, D., Medrano, G. A., Bisteni, A., and de Michelli, A.: The Electrograms of the Conductive Tissue in the Normal Dog's Heart. Am. J. Cardiol. 4: 459 (Oct.), 1959.

Unipolar electrocardiograms were obtained in the dog from the atrioventricular node, the bundle of His, the right and left bundle branches, and from septal Purkinje fibers via a tiny needle electrode inserted precisely into the respective areas. The wave forms, as expected, appeared in the appropriate portion of the P-R segment. The speed of conduction between the bundle of His and the right branch was calculated to be 2,656 mm. per second while that between the left branch and septal Purkinje fibers was 2,107 mm. per second. The right and left bundle branches were found to be activated simultaneously. Tracings obtained from each area are depicted and described. Nomenclature is proposed and problems for investigation by this technic are considered.

ROGERS

Sotgiu, G., and Tumiotto, G.: Electrocardiographic Modifications after Administration of Glucose. Acta cardiol. 14: 284, 1959.

In 431 subjects electrocardiograms were recorded 30 minutes after the ingestion of a 150-Gm. dose of glucose. Of these patients 31 were normal and the majority of the others had heart disease. In the normal subjects the glucose test showed no electrocardiographic changes. In the patients with heart disease, 6 per cent showed some modification in the electrocardiogram, consisting of a reduction or modification of the T wave, which disappeared when ATP was given. The authors assumed that these electrocardiographic alterations were related to preexisting ischemic conditions and that the changes following glucose ingestion were due to a decrease of phosphorylation and cellular oxidation.

BRACHFELD

Van Houte, O., and Sternon, J.: Contribution to the Study of Juvenile Left Ventricular Hypertrophy. Acta cardiol. 14: 294, 1959.

The criteria usually accepted for the electrocardiographic diagnosis of early left ventricular hypertrophy are discussed. Statistics covering

1,000 patients, between the ages of 18 and 25, are presented, and attention is drawn to the absence of a significant concordance between the fulfillment of the Sokolow criteria and the existence of pathologic left ventricular hypertrophy. The authors emphasize the difficulties in diagnosing early left ventricular hypertrophy in young adults.

BRACHFELD

Weitzman, D., and Smith, E. B.: Ischaemic Electrocardiograms in Symptomless Men. Brit. M. J. 2: 162 (Aug. 15), 1959.

Ischemic electrocardiograms were identified in 12 instances of tracings recorded on 100 hospitalized men between the ages of 50 and 80. In each instance, the presence of clinical evidence of heart disease (either in the past or current) was carefully excluded. The electrocardiographic evidences of myocardial ischemia were large Q waves, flattening or inversion of T waves, and depression of the S-T segment at rest or after exercise. Although these changes in the electrocardiogram are not specific for myocardial ischemia all other possible causes were excluded. The authors reemphasize the need for care in using electrocardiography in the assessment of chest pain which is not necessarily of cardiac origin even though the tracing be abnormal. In 59 of these men, including 9 with abnormal tracings, the serum lipoproteins were examined by paper electrophoresis for the presence of an abnormal prebeta-lipid band (which corresponds approximately with the Sf 20-100 fraction of Gofman et al.). The results were compared with those of known cases of ischemic heart disease. A moderate quantity of abnormal lipid was found in a similar percentage of both groups and did not correlate with clinical coronary disease. A large abnormal band did, however, appear to correlate with electrocardiographic evidence of myocardial ischemia.

KRAUSE

Wolff, L.: Diagnostic Clues in the Wolff-Parkinson-White Syndrome. New England J. Med. 261: 637 (Sept. 24), 1959.

Three cases are presented as illustrations of the fact that the Wolff-Parkinson-White syndrome is still frequently misdiagnosed initially and that serious consequences may result from an incorrect diagnosis. It is pointed out that this condition should be considered whenever the P-R interval measures 0.12 second or less, in every case of paroxysmal tachycardia and whenever supraventricular tachycardia is promptly interrupted after procaine amide. Since Q-S deflections often occur in certain leads when anomalous conduction is present in the absence of myocardial infarction, an electrocardiographic or vectorcardiographic diagnosis of myocardial infarction cannot be made (nor excluded) in the presence of anomalous excitation.

SAGALL

ENDOCARDITIS, MYOCARDITIS, AND PERICARDITIS

Engle, R. L., Jr., and Koprowska, I.: The Appearance of Histiocytes in the Blood in Subacute Bacterial Endocarditis. Am. J. Med. 26: 965 (June), 1959.

A patient with subacute bacterial endocarditis whose peripheral blood contained large numbers of histiocytes phagocytizing all the other blood elements is reported. The bizarre cells were seen particularly in smears obtained from the ear lobe. After adequate antibiotic therapy, they disappeared. The cells were of 2 types. First, there were large cells measuring up to 20 by 30μ in diameter and usually containing cytoplasmic inclusions. The nuclei were large, oval or round, and occasionally indented. A single, very large, distinct nucleolus was often present. Cytoplasm was abundant and finely granular. The second type of cell was smaller and appeared to be an intermediate form between the first type of cell and a typical monocyte. Its nuclei had a less reticular chromatin pattern, and were frequently lobulated or kidney shaped. The cytoplasm had moderate numbers of granules.

RINZLER

Zinsser, H. F., Blakemore, W. S., Kirby, C. K., Johnson, J.: Invalidism due to Recurrent Idiopathic Pericarditis with Recovery after Pericardiectomy. J.A.M.A. 171: 274 (Sept. 19), 1959.

Recurrent idiopathic pericarditis can cause a form of invalidism different from that usually seen in chronic pericardial constriction or tamponade. The recurrent chest pain, fever, and malaise result in a severe disability. It is often characterized by multiple hospitalizations, inability to work regularly, and severe financial hardship. In 8 cases reported, various forms of medical treatment did not relieve the symptoms. In all cases recovery ensued after as much as possible of the diseased pericardium was removed through a sternum-splitting incision and the patients returned to work.

KITCHELL

HYPERTENSION

Atanassowa, M.: Blood Pressure Changes Caused by Meterologic Factors. Ztschr. Kreislaufforsch. 48: 461 (May) 1959.

In 1953 the number of new hypertensive cases among university students was twice as great as in the preceding and following years. This was attributed to the fact that 1953 was characterized by exceptionally great and sudden temperature changes. The average blood pressure of students was highest during periods of sudden changes in temperature, barometric pressure, and atmospheric electricity. This was the case in February more than in any other month of 1957. The morbidity of hypertension seemed to be higher in those sections of Bulgaria that have a continental climate.

LEPESCHKIN

Bartels, C. C., Evans, J. A., and Townley, R. G.: Chlorothiazide—A Survey of its Effects in Hypertensive Patients. J.A.M.A. 170: 1796 (Aug. 8), 1959.

A hypotensive effect from chlorothiazide was noted in 80 per cent of 65 patients studied. This effect was most marked in those with a severe hypertension and in those receiving ganglionic blocking agents. Of 23 patients previously requiring ganglionic blocking agents, 12 were able to discontinue that therapy, and 6 more were able to reduce the dosage to one half or less when chlorothiazide was added. Twenty-eight patients showed a drop in serum potassium to 3.5 mEq. or less. This occurred in 12 patients, despite concurrent administration of 2 to 3 Gms. of potassium daily. Renal lesions may accompany potassium deficiency but in this series depletion occurred with and without impaired renal function. Periodic determinations of serum potassium seem indicated for those on long-term chlorothiazide treatment. Caution should be taken to interrupt the drug administration when diarrhea or vomiting occur, as these could cause severe potassium depletion.

KITCHELL

Cohen, A. S., Stearns, N. S., Levitin, H., and Hurwitz, D.: Studies on Rauwolfia Alkaloids in Diabetic Hypertensive Patients. Ann. Int. Med. 51: 238 (Aug.), 1959.

The effects of reserpine upon diabetes mellitus and hypertension were studied in 27 women and 1 man with proved diabetes mellitus and with hypertension for at least 5 years. Dietary management was sufficient to control the diabetes in 10 patients; the remaining 18 patients required insulin in addition to dietary control. The final dose of reserpine was adjusted for each individual patient until a hypotensive effect occurred or side effects appeared. Twenty-five of the 28 patients had symptoms such as headache, fatigue, dizziness, tinnitus, and epistaxis prior to therapy. In 18 of these 25 patients improvement in these subjective symptoms was noted during treatment (1 to 12 months). The remaining 7 patients experienced no symptomatic change. A fall in mean arterial blood pressure of 20 mm. Hg or greater occurred in 14 of 28 patients; 7 others showed a fall of 10 to 17 mm. Hg. Of 18 patients initially receiving insulin, the dosage was unchanged in 12, decreased in 5, and increased in 1 (30 units). No change occurred in the diabetic status of the 10 patients who had been controlled on diet alone. No significant weight changes occurred in the patients during the study. A second group of 10 hypertensive diabetic patients was followed in a controlled, double-blind study of the effects of the alseroxylon fraction of the crude Rauwolfia serpentina root. In this smaller group all patients were on long-acting insulin preparations. There was no change in either the diabetic status or the weight in the patients. The mean arterial blood pressure appeared to fall during the administration of the Rauwolfia preparation.

KAYDEN

Gibson, G. B., and Platt, R.: Incidence of Hypertension after Pregnancy Toxaemia. Brit. M. J. 2: 159 (Aug. 15), 1959.

This study was undertaken in an attempt to clarify the effect of toxemia of pregnancy on the level of blood pressure in the woman who enters her pregnancy with a normotensive blood pressure or with hypertension. The authors noted that their control group was from an unselected population and should have ideally been a series of women who had passed through a nontoxemic pregnancy. A review of 446 cases of preeclampsia in women whose blood pressure early in pregnancy was normal showed that their mean blood pressure 4 years later was much higher than the expected mean of an unselected population. Nevertheless, severe hypertension was rare. Similarly, in a woman who had her toxemia superimposed on hypertension, the rise of blood pressure on reexamination was more than could be accounted for by an increase of age alone.

KRAUSE

Gillespie, L., Jr., Terry, L. L., and Sjoerdsma, A.: The Application of a Monoamine-Oxidase Inhibitor, 1-Phenyl-2-Hydrazinopropane (JB-516), to the Treatment of Primary Hypertension. Am. Heart J. 58: 1 (July), 1959.

The observation that patients receiving iproniazid (Marsilid) frequently developed postural

hypotension led to the investigation of the hypotensive effect of various monoamine-oxidase drugs. A preliminary evaluation of the monoamine-oxidase inhibitor, 1-phenyl-2-hydrazinopropane (JB-516, Catron) in 21 hypertensive patients revealed orthostatic lowering of the blood pressure in 18 and a distinct lowering of the recumbent blood pressure in 6. The effects of the drug simulated those produced by sympathectomy and ganglionic blocking agents but were not associated with the parasympatholytic effects commonly observed with the latter group of drugs. In 6 patients a loss of red-green color discrimination without other visual defects occurred with large doses of JB-516, but subsided promptly on discontinuance of the drug. Chlorothiazide in several patients appeared to potentiate the antihypertensive effect of JB-516. Evidence of inhibition of monoamine-oxidase in man by JB-516 is presented, and attention is called to a possible relationship between the inhibition of this enzyme and the reduction of blood pressure.

SAGALL

Goldstein, S., and Rossi, G. V.: Potentiation of the Activity of Hypotensive Drugs: Effect of Several Synthetic Compounds on Six Hypotensive Agents in Hypertensive Rats and Normotensive Dogs. J. Pharmacol. & Exper. Therap. **126:** 168 (June), 1959.

Four compounds of diverse structure were selected for study because they enhanced markedly the action of certain drugs in doses that alone exerted little or no pharmacologic effect. These compounds were (1) beta-diethylaminoethyl diphenylpropyl acetate HCL (SKF 525-A); (2) 2, 4 - dichloro-6-phenylphenoxyethyl diethylamine HBr (Lilly 18947); (3) 2 - (1-phenylethyl)-4-pentenamide (NDR A-1358), and (4) 2-(p-chlorobenzyl)-4- pentenoic acid hydrazide (NDRA-2435). These 4 compounds were shown to enhance markedly the intensity and duration of the depressor response to reserpine, rescinnamine, hydralazine, mecamylamine, 2-(N, N-diallylcarbamylmethyl)-aminomethyl-1, 4-benzodioxan, and mannitol hexanitrate in unanesthetized chronic hypertensive rats. Subthreshold doses of the 6 hypotensive drugs produced a significant reduction of systolic blood pressure when administered to hypertensive rats pretreated with the 4 synthetic compounds. When the injection of the potentiating agents was made after the blood pressure reducing activity of the hypotensive drugs had largely terminated, there was a renewed and markedly increased depressor response in hypertensive rats. Essentially the same findings were obtained in unanesthetized normotensive dogs.

RINZLER

Mallov, S.: Comparative Reactivities of Aortic Strips from Hypertensive and Normotensive Rats to Epinephrine and Levarterenol. Circulation Research **7:** 196 (Mar.), 1959.

The hypothesis that vascular hyperactivity may be the basis of some forms of hypertension was examined by use of aortic muscle strips obtained from rats rendered hypertensive by experimental means. Aortic strips from DCA-salt hypertensive rats and renal hypertensive rats responded to various concentrations of epinephrine and levarterenol with less isometric tension than did the corresponding control aortic strips from normotensive rats. The decreased responsiveness of the hypertensive strips may be one of the consequences of the hypertensive state, and the authors suggest that these results do not support the hypothesis that vascular hyperactivity may be the basis of hypertension.

PAUL

Prado, J. L., and Carlini, E. A.: Influence of Tetraethylammonium, Pentolinium and Hexamethonium on the Action of Hypertension. Arch. int. pharmacodyn. **122:** 100 (Oct.), 1959.

Tetraethylammonium increased 2 to 3 fold the smooth muscle stimulating effect of hypertension on isolated guinea-pig ileum, rat uterus, rat hindquarter vessels, and the blood pressure of the pithed rat. This augmenting effect could not be ascribed to the ganglion blockade itself. The potentiating doses of tetraethylammonium were higher than those necessary to block stimulating doses of DMPP (1, 1-dimethyl-4-phenylpiperazinium) and neither pentolinium nor hexamethonium blockade produced the same augmenting effect, even when the doses of these agents were much higher than the blocking doses. The mechanism of the synergism of tetraethylammonium and hypertensin remains unknown.

BRACHFELD

Schwartz, C., Stechel, G. H., Fishman, S. I., and Fankhauser, A.: Clinical Therapeutic Evaluation of Hydrochlorothiazide (Hydrodiuril) J.A.M.A. **170:** 2057 (Aug. 22), 1959.

In most patients treated earlier with chlorothiazide, maintenance of the previous status was obtained with a dose of hydrocholorothiazide one tenth that of cholorothiazide. Hypertensive patients without clinical congestive failure showed improvement symptomatically and by fall in blood pressure. Where necessary, intravenous therapy with hydrocholorothiazide is convenient. An important advantage appears to be the lack of weakness in patients receiving hydrochlorothiazide.

KITCHELL

METABOLIC EFFECTS ON CIRCULATION

Dawes, G. S., Mott, J. C., and Shelley, H. J.: The Importance of Cardiac Glycogen for the Maintenance of Life in Foetal Lambs and Newborn Animals during Anoxia. J. Physiol. **146**: 516 (June 11), 1959.

Anoxia was produced by ligation of the umbilical cords of fetal lambs during continuous recording of the blood pressure and heart rate. Although adult lambs were unable to survive complete anoxia for longer than 7 minutes, the fetal lambs of 83 to 91 days gestation age were able to survive for up to 60 minutes. In the fetal lambs the vital signs returned completely to normal when anoxia was halted within 40 minutes, whereas lambs of 126 to 146 days gestation age maintained their vital signs for only 10 to 15 minutes after the onset of anoxia. The initial rates of glycolysis in the tissues of the older lambs were either equal to or greater than those in the younger lambs, and a correlation was observed between the survival time and the initial cardiac carbohydrate concentration in the lambs of each age group. In newborn rats, guinea pigs, and rabbits there was a linear relationship between cardiac carbohydrate concentration and the predicted time of survival in 100 per cent nitrogen. The authors conclude that the maintenance of the circulation is of paramount importance in determining survival in animals subjected to anoxia.

KARPMAN

Goodale, W. T., Olson, R. E., and Hackel, D. B.: The Effects of Fasting and Diabetes Mellitus on Myocardial Metabolism in Man. Am. J. Med. **27**: 212 (Aug.), 1959.

Myocardial metablism was studied by cardiac catheterization in normal human subjects, fed and in the fasting state, and in patients with mild diabetes mellitus. There was a direct dependence of myocardial extraction on arterial concentration. Thus, in the fasting state, arterial levels and cardiac extraction of glucose, lactate, and pyruvate were reduced. For each substrate, there was a definite threshold of myocardial utilization. In the fasting state, a small A-V glucose difference accounted for 10 to 32 per cent of myocardial oxygen utilization, but after a light meal, glucose accounted for 50 to 80 per cent. During fasting, carbohydrate extraction decreased, the respiratory quotient approached 0.7, and the heart appeared to subsist mainly on fat, but postprandial carbohydrate extraction accounted for the bulk of cardiac energy requirement. Myocardial oxygen extraction (A-V/A) was remarkably constant in all subjects and varied linearly with arterial oxygen level from mild anemia to marked polycythemia. In the diabetic patient myocardial glucose pyruvate and lactate extraction was depressed. Postprandially, there was a rise in extraction, but this occurred at such high arterial levels that extraction coefficients were still depressed. With the administration of insulin, there was a pronounced rise in myocardial carbohydrate extraction coefficients to normal and a rise in myocardial respiratory quotient.

KURLAND

Pines, I., Salazar, E., and Lopez, T.: On the Influence of the Intravenous Injections of Gluco corticoids upon the Electrocardiogram. An Experimental Study. Arch. int. pharmacodyn. **122**: 1 (Oct.), 1959.

The effect of the intravenous or intracardiac injections of prednisolone phosphate was studied in normal anesthetized dogs with the help of electrocardiograms as well as endoelectrograms derived from the upper third of the right atrium. Ventricular premature beats and ventricular bigeminy were nearly constantly observed after doses of about 15 mg. per Kg. Intravenous injections of chlorothiazide, chloroquine, and moderate amounts of digitalis prevented or suppressed these ventricular ectopic rhythms. These beats belonged apparently to the first type, or nondigitalis ventricular extrasystoles and depended probably on the direct enhancement by the gluco-corticoids of the excitability of the ventricular ectopic centers. Injections of glucosteroids to patients prone to develop ventricular arrhythmias, as for instance after myocardial infarctions, should be given with caution. The disturbance of the intraventricular conduction observed in some experiments could not be explained and did not tally with the improvement in atrioventricular conduction observed clinically with these steroids.

BRACHFELD

Spurr, G. B., and Barlow, G.: Influence of Prolonged Hypothermia and Hyperthermia on Myocardial Sodium, Potassium and Chloride. Circulation Research **7**: 210 (Mar.), 1959.

Myocardial electrolyte and water contents were determined by direct analysis of left ventricular muscle in dogs cooled to 25 C. In animals maintained at this rectal temperature a progressive increase in intracellular potassium and a decrease in myocardial sodium content, with no change in total chloride and water were noted. Induction of hyperthermia to a rectal temperature of 41.5 C

resulted in a rise in content of left ventricular potassium, with no significant alterations in total sodium, chloride, or water contents. The hypothermic animals exhibited increased myocardial excitability. The relation of the electrolyte changes, however, to the increased excitability remains uncertain.

PAUL

Zarafonetis, C. J. D., Seifter, J., Baeder, D., Kalas, J. P., and Chang, W. Y. M.: Inhibition of Lipid Mobilization of Surgical Stress by Partially Depolymerized Hyaluronic Acid. Am. J. M. Sc. **237**: 771 (June), 1959.

The authors have previously demonstrated that in response to caloric requirements induced by the stress of surgery, lipid mobilizer hormone (LM) was released from the posterior pituitary gland into the circulation. This substance then caused a mobilization of triglyceride from the omentum into the portal circulation. In addition the omentum appeared to remove some cholesterol from the blood circulating through it. Changes in peripheral blood following surgical stress reflected these events plus the capability of the liver to cope metabolically with the lipid load presented to it. Various substances have been found to show a lipemia clearing effect. Of these, a partially depolymerized hyaluronic acid (PDHA) was selected because it was nontoxic and effective by oral administration. The effect of PDHA on the prehepatic and peripherally circulating blood lipids was studied in 9 females during the course of an intraabdominal operation. There was a marked increase in circulating endogenous LM hormone during the course of surgery. This was not followed by an outpouring of fatty acids into the prehepatic circulation as was uniformly exhibited by patients not pretreated by PDHA. Thus, PDHA did not prevent the release of LM from the posterior pituitary, but did apparently prevent the effect of LM at the omental levels. There were no untoward clinical effects. PDHA did not display any anticoagulant action. The mode of action of PDHA in preventing the omental fat depot from responding to lipid mobilizer hormone is not known at present.

SHEPS

PATHOLOGY

Caulfield, J. B., and Klionsky, B.: Myocardial Ischemia and Early Infarction; an Electron Microscopic Study. Am. J. Path. **35**: 489 (May-June), 1959.

Myocardial ischemia was produced in rabbits by ligation of the coronary arteries. As early as 5 minutes after ligation, glycogen disappeared from the ischemic myocardium and structural changes (characterized by clumping of nucleoplasm and cytoplasmic distortion) became manifest. Ischemia continuing for 30 minutes was sufficient to result in loss of contractible capacity and cell death but the actual disintegration of muscle fibers proceeded at a rate that was apparently dependent upon the activity of the muscle during the ischemic period. Electron microscopy clearly demonstrated continuity of the sacrolemmal membrane of heart muscle and its endoplasmic reticulum; this was thought to provide a morphologic basis for the conduction of surface potentials throughout the cardiac muscle fiber.

KARPMAN

Heath, D., Wood, E. H., DuShane, J. W., and Edwards, J. E.: The Structure of the Pulmonary Trunk at Different Ages and in Cases of Pulmonary Hypertension and Pulmonary Stenosis. J. Path. & Bact. **77**: 443 (Apr.), 1959.

The pulmonary trunk and aorta were examined histologically in 71 control subjects, in 18 patients with acquired pulmonary hypertension, in 26 cases of congenital cardiac defects with pulmonary hypertension, in 12 cases of pulmonary stenosis, and in 19 patients with Fallot's tetrad. In the normal individual with a high intravascular pressure (as in the aorta) the elastic fibrils are long, uniform, and arranged circumferentially in a parallel fashion; when the pressure is low (as in the pulmonary artery) the fibrils are short and irregular in shape and configuration. In fetal life the pulmonary arteries show a high pressure configuration, which changes to the low pressure appearance in adulthood. Patients with congenital heart disease and pulmonary hypertension may retain the fetal pattern (if the hypertension is congenital) or may undergo the transition to the adult pattern (if the hypertension is acquired); patients with acquired heart lesions and pulmonary hypertension reveal the adult pattern. The thickness of the media of the pulmonary trunk is equal to that of the aorta in the fetus but is only 40 to 70 per cent as thick in the adult; in severe congenital or acquired pulmonary hypertension, the media of the pulmonary artery and aorta is equal even in the adult. In pulmonary stenosis the inactivity of the pulmonary artery is thought to produce the thinning of the media associated with thin, sparse elastic fibrils. Patients with Fallot's tetrad reveal the fetal configuration of the media. The authors conclude that the lessor circulation, (i.e. the right ventricle, the elastic major arteries, and the small muscular pulmonary arteries) may be considered as a con-

tinuous tube of muscle that exhibits characteristic reactions to changes in intravascular pressure. They propose that the term "tetralogy of Fallot" be eliminated in favor of functionally descriptive terms describing the degrees of the ventricular septal defect and the pulmonary stenosis.

KARPMAN

Reiner, L., Rodriguez, F. L., Platt, R., and Schlesinger, M. J.: Injection Studies of the Mesenteric Arterial Circulation. I. Technique and Observations on Collaterals. Surgery 45: 820 (May), 1959.

A technic of postmortem study of the celiac and the superior and inferior mesenteric circulations is described. The abdominal viscera were removed en bloc. Then, a gelatin-barium sulfate mass was injected into each of these arteries under 200 mm. Hg pressure for as long as 15 minutes. The specimen was separated into 3 parts representing the 3 circulations. Roentgenograms clearly demonstrated each patent artery down to a diameter of 40 μ. Dissection with scissors showed the nature of obstructions. Injection of any 1 of the 3 principal arteries generally filled the arborizations of the other 2, indicating good collateral filling of the normal viscera. Ordinarily, ligation of up to 3 primary or subsidiary jejunal arteries did not prevent filling of their arborizations. However, ligation of vasa recta to the upper jejunum frequently caused serious filling defects, despite the rich anastomosis of their branches within the intestinal wall. Some adipose arteries were found capable of contributing significantly to the collateral supply of the mesenteric circulation.

ROGERS

Spreer, F.: On a Quantitative Non-distorting Method of Obtaining Cardiac Cavity Molds. I. Methods. Changes in the Shape of the Cavities of Contracted and Relaxed Hearts. Ztschr. Kreislaufforsch. 48: 501 (June), 1959.

During autopsies performed 5 to 12 hours after death, hearts that were either in complete rigor or completely relaxed were injected in situ, without disturbing the pericardium, with a mixture of 3 parts dental plaster of Paris and 2 parts 5 per cent formol, which did not harden until after 15 minutes and left the myocardium undamaged for future studies. In order to simulate conditions of a natural systole in hearts in rigor, the right ventricle was filled at a pressure of 30 mm. Hg and the left ventricle at a pressure of 100 mm. Compared to the relaxed ventricle, the contracted ventricle showed an almost complete disappearance of interpapillary spaces, while the outflow tract was shorter and rounder; in the right ventricle it was more curved around the crista supraventricularis. Many other peculiarities could be disclosed by this method of study.

LEPESCHKIN

PHARMACOLOGY

Arora, R. B., and Sharma, P. L.: Effect of Quinidine and Tranquilizing Agents on Ectopic Ventricular Tachycardia. Lancet 1: 962 (May 9), 1959.

Two-stage ligation of the anterior descending branch of the left coronary artery in dogs produces a long-lasting ventricular tachycardia. Mixtures of quinidine and various tranquillizing agents were tested for their ability to suppress ventricular ectopic beats in these animals. Mixtures of quinidine and benactyzine or alseroxylon produced potentiative synergism. No synergism was noted with chlorpromazine and quinidine, and reserpine actually antagonized the action of quinidine. It is suggested that such mixtures may be of use in arrhythmias that follow myocardial infarction in man.

SHEPS

Bär, C. G., and Bachmann, K.: Changes in Central Blood Volume under the Influence of Ganglionic Blockade and their Recognition by Means of Dye Dilution Curves. Ztschr. Kreislaufforsch. 48: 529 (June), 1959.

In 14 persons with arterial hypertension and increased cardiac output Geigy blue was injected into a cubital vein. The peripheral dye concentration was then recorded by means of an ear oxymeter, before and after injection of 3 mg. of Camphidonium. The cardiac output and the stroke volume (determined according to Broemser and Ranke) showed a decrease parallel to a decrease of central blood volume, which manifested itself as a decrease in the time of passage of the dye (interval between the beginning of the descending branch and end of the ascending branch of the light transmission curve). The circulation velocity (determined from the time of appearance of the descending branch after injection) showed no significant changes. The decrease in the central (pulmonary) blood volume was attributed to pooling of blood in the splanchnic region. The dye dilution method was simpler and less unpleasant for the patient than other methods capable of demonstrating these changes.

LEPESCHKIN

Berry, J. N., Thompson, H. K., Jr., Miller, E., and McIntosh, H. D.: Changes in Cardiac Output Stroke Volume, and Central Venous Pressure Induced by Atropine in Man. Am. Heart J. **58:** 204 (Aug.), 1959.

Observations following the intravenous administration of atropine in 22 recumbent subjects confirmed previous reports that this procedure results in an increase in cardiac output, a fall in stroke volume, and a decrease in central venous pressure. In addition, it was found that the maximum increase in cardiac output occurred within 3 minutes after the drug was given. During this early period there was no change in stroke volume. Following this 3-minute interval there was a relative decline in cardiac output with no change in heart rate, resulting thereby in a significant fall in stroke volume. Certain maneuvers intended to increase venous return (hyperventilation, passive leg-raising, and head-down tilting) failed to increase cardiac output or stroke volume after the injection of atropine, whereas, in contrast, the rapid infusion of 50 Gm. of serum albumin produced a marked increase in minute volume and stroke volume after atropinization. Several hypotheses explaining the circulatory changes produced by atropine are suggested and discussed.

SAGALL

Boura, A. L. A., Green, A. F., McCoubrey, A., Laurence, D. R., Moulton, R., and Rosenheim, M. L.: Darenthin: Hypotensive Agent of New Type. Lancet **2:** 17 (July 11), 1959.

Extensive pharmacologic studies in animals and man of Darenthin (bretylium tosylate), a new hypotensive agent, are reported. The drug is one of the series of benzyl quaternary ammonium compounds that selectively block the peripheral sympathetic (adrenergic) nervous system without antagonizing the effects of released or injected epinephrine or norepinephrine. With this drug there is no depression of the parasympathetic nervous system and no central depression. This blocking effect is related to a selective accumulation of the drug in the adrenergic neurons—peripheral to the ganglionic synapsis. Darenthin was used to treat 36 patients with moderate to severe hypertension over a period of 1 to 26 weeks. The initial oral dose was 70 to 1[illegible] mg. of base 3 times a day, and gradually increased as necessary. The eventual treatment dose varied from 130 to 840 mg. of base 3 times a day. There was a moderate fall in supine blood pressure and a pronounced postural drop. This started 1½ to 3 hours after administration of the drug and lasted about 8 hours. It seemed likely that tolerance occurs in man. Chlorothiazide was added with apparent potentiation of the hypotensive effect. Side effects were trivial and attributable to sympathetic blockade. Stuffy nose occurred more often in normotensive volunteers than in hypertensive patients. Occasional dropped beats were noted in a few subjects. Since norepinephrine and epinephrine have a greater pressor effect following Darenthin, and since (unlike the ganglion-blocking agents) Darenthin did not block the mechanism of release of these catecholamines from the adrenal medulla, the activity of the adrenal medulla may be expected to influence blood pressure and various sympathetic functions to a relatively greater extent after administration of this compound. Very severe hypertension developed in a patient with pheochromocytoma after the cautious intravenous administration of 53 mg. of base. On the other hand, norepinephrine may readily be used to counteract excessive hypotension produced by Darenthin.

SHEPS

Chappel, C. C., Rona, G., Balazs, T., and Gaudry, R.: Severe Myocardial Necrosis Produced by Isoproterenol in the Rat. Arch. int. pharmacodyn. **122:** 123 (Oct.), 1959.

Isoproterenol was given to groups of rats in a single injection of lethal and sublethal doses, or on 2 consecutive days at lower dosage levels. Survivors were autopsied after 48 hours. Dosages ranged from 680 mg./Kg. (LD_{50}) to 0.02 mg./Kg. Animals receiving doses greater than ¼ the LD_{50} showed severe lesions of the lung, brain, liver, and kidney, consistent with the picture of severe shock. These lesions were characterized by pulmonary edema and hemorrhage, hydrothorax, meningeal congestion, cerebral edema, congestive centrolobular hepatic necrosis, and ischemia or necrosis of the renal cortex. At lower doses congestive lesions were observed in only the abdominal viscera and at 0.33 mg./Kg. no extracardiac lesions were found. Dosages of 0.08 mg./Kg. or greater caused macroscopic cardiac lesions often localized to apex and papillary muscle; these lesions were extensive and infarct-like at lower doses. It has been reported that isoproterenol administered intravenously to the dog caused a marked fall in blood pressure and increased the rate and amplitude of the heart beats. These pharmacologic actions would tend to increase the oxygen requirements of the heart muscle and at the same time reduce the available supply. Since the authors found that doses as low as 1/500 of the LD_{50} caused gross and microscopic myocardial necrosis it would appear

likely that these lesions are secondary to the cardiovascular effects of the drug rather than a direct toxic effect on the myocardium.

BRACHFELD

Church, G., and Marriott, H. J. L.: Digoxin: Single Versus Divided Daily Maintenance Dosage. Am. Heart J. **58**: 171 (Aug.), 1959.

A short clinical study on 30 outpatients showed that some patients could be satisfactorily maintained without evidence of toxicity on a higher daily dose of digoxin when it was given in divided doses rather than in a single dose. These findings were in contrast to the reports in the literature advocating a single daily dose of digoxin as the most satisfactory way of administering the drug. The authors concluded that in the majority of patients minimal toxicity and optimal maintenance with digoxin can best be achieved by giving the daily dose in divided amounts.

SAGALL

Cox, E., and Wright, S. E.: The Hepatic Excretion of Digitalis Glycosides and their Genins in the Rat. J. Pharmacol. & Exper. Therap. **126**: 117 (June), 1959.

Paper chromatography and colorimetric methods were used to follow and compare the excretion of some digitalis glycosides and their genins in the bile of rats. In addition the effect of chemical constitution on the excretory pattern was noted. In bile lanatosides A and C were excreted unchanged. Digitoxin, digoxin, and digoxigenin were present partly unaltered and partly as metabolites that still retained the unsaturated lactone ring. Digitoxigenin was excreted entirely as metabolites. The amount of glycoside or aglycone and their metabolites present in the bile within 5 hours of glycoside administration ranged from 70 to 80 per cent of the dose for lanatosides A and C to about 10 per cent for digitoxin. About 15 per cent of the dose of each aglycone was excreted in bile.

RINZLER

David, F., Nash, C. B., and Woodbury, R. A.: Cardiovascular Effects of Anesthetic Doses of Thiopental Sodium. Anesth. & Analg., Current Researches **38**: 222 (May-June), 1959.

Ten dogs were maintained in the upper level of plane 2 of stage III anesthesia with thiopental sodium for a period of 4 hours. A significant reduction of the cardiac index (determined by the pressure contour method of aortic pressure registered with a Hamilton manometer) occurred 45 minutes after induction of anesthesia, with a slower decrease continuing thereafter. No serious change was observed in the mean blood pressure. The heart rate was markedly accelerated immediately after induction of anesthesia, and remained so during the period of anesthesia. The plasma volume, the total blood volume, and the value for plasma protein were diminished.

LEPESCHKIN

Dresdale, D. T., Yuceoglu, Y. Z., Michtom, R. J., Schultz, M., and Lunger, M.: Effects of Lanatoside C on Cardiovascular Hemodynamics. Acute Digitalizing Doses in Subjects with Normal Hearts and with Heart Disease without Failure. Am. J. Cardiol. **4**: 88 (July), 1959.

Cardiovascular activities before and for periods up to 2 hours after the intravenous injection of 1.2 to 1.6 mg. of lanatoside C were observed by right heart catheterization in 7 healthy adults and in 4 persons having heart disease without failure or marked cardiomegaly. No significant change was found in heart rate, cardiac output, systemic or pulmonary arterial pressure, right ventricular systolic and systemic venous pressures, and arteriovenous oxygen difference. Right atrial or right ventricular end diastolic pressures fell slightly in some patients. It was concluded that this type of acute digitalization has little detectable hemodynamic effect and that no deleterious consequences are likely to result from prophylactic digitalization in patients such as these.

ROGERS

Edmonds, C. J., and Wilson, G. M.: Hydroflumethiazide. Lancet **2**: 303 (Sept. 12), 1959.

Hydroflumethiazide is a new diuretic that differs from chlorothiazide in that the thiadiazine ring is saturated and the chlorine atom is replaced by a trifluoromethyl group. Its action was studied in 2 normal men and 30 patients. In normal subjects, it considerably increased sodium excretion up to 10 hours after administration, a duration of action considerably longer than chlorothiazide. The most characteristic difference from chlorothiazide was in the increased bicarbonate excretion produced by the latter. In effective clinical trial with 50 mg. 3 times a day, diuresis began as soon as treatment was started and continued until edema was gone. In 7 patients with pulmonary heart disease, a good result was achieved in only 3, in contrast to good results in 8 out of 10 with ischemic heart disease, in all 3 patients with thyrotoxicosis and heart failure, and in 6 of 7 with chronic rheumatic heart

disease. No effect was noted in 1 patient with nephrosis and 1 with hepatic cirrhosis and ascites. Hypokalemia occurred in 7 patients and was corrected by 3 Gm. of potassium chloride daily. Hydroflumethiazide was at least 10 times as effective by weight as chlorothiazide, but the maximal rate of sodium excretion was the same.

KURLAND

Emmelin, N.: Supersensitivity due to Prolonged Administration of Ganglion-Blocking Compounds. Brit. J. Pharmacol. **14:** 229 (June), 1959.

The effect of repeated injections of hexamethonium, of chlorisondamine (Ecolid) and of an atropine-like drug (piperidino-ethyl-diphenyl-azetamid) in sensitizing the cat's salivary gland and nictitating membrane to epinephrine was studied. Chlorisondamine administration for 1 to 3 weeks induced supersensitivity in normally innervated structures almost equal to that achieved by denervation. Hexamethonium administration failed to sensitize the gland until it has been renervated surgically by cross-suturing. Acute administration of these drugs did not affect the epinephrine sensitivity of the normal structures. Cannon's law that postganglionic nerve section causes a more pronounced sensitivity than preganglionic section thus seemed applicable when the denervation was produced pharmacologically.

ROGERS

Fleming, P. R., Zilva, J., Bayliss, R. I. S., and Pirkis, J.: Hydrochlorothiazide: A Comparison with Chlorothiazide. Lancet **1:** 1218 (June 13), 1959.

Hydrochlorothiazide was compared with chlorothiazide in healthy young subjects, convalescent patients, and in patients with edema. In the first 2 groups hydrochlorothiazide was 20 times more potent than chlorothiazide. In patients with edema this relationship varied from 5 to 10 times. This greater potency may have been related to better intestinal absorption and to the fact that hydrochlorothiazide was not excreted in the bile and hence there was more available for renal elimination. Hydrochlorothiazide did not promote the exeretion of bicarbonate to the same degree as does chlorothiazide. There was little difference in the promotion of potassium excretion. It is therefore suggested that whenever hydrochlorothiazide is given on a continuous basis supplements of potassium chloride should be given.

SHEPS

Havard, C. W. H., and Fenton, J. C. B.: Clinical Evaluation of Hydrochlorothiazide. Brit. M. J. **1:** 1568 (June 20), 1959.

Hydrochlorothiazide was compared to chlorothiazide in 21 edematous patients. A dose of 100 mg. of hydrochlorothiazide produced a quantitative and qualitative response similar to that of 1,000 mg. of chlorothiazide. In 3 patients, hydrochlorothiazide seemed to produce a more satisfactory diuresis. In vitro hydrochlorothiazide was less inhibitory than chlorothiazide to carbonic anhydrase but the diuretic action appeared to be similar and potassium loss in the urine occurred just as readily with the use of hydrochlorothiazide. It is pointed out that patients with chronic edema and sodium retention are particularly likely to lose considerable quantities of potassium in the urine and are especially liable to hypokalemia with the use of these drugs.

KRAUSE

Johnston D. H., and Cornish, A. L.: Acute Pancreatitis in Patients Receiving Chlorothiazide. J.A.M.A. **170:** 2054 (Aug. 22), 1959.

Acute pancreatitis was diagnosed in 3 women, all 50 years of age or older, who had been taking 0.5 to 1.0 Gm. of chlorothiazide daily for 2 or 3 months for therapy of cardiovascular disease. There was no history of conditions considered as predisposing to acute pancreatitis. Although the specific etiology remained undetermined, chlorothiazide was considered as a possible cause.

KITCHELL

Jourdan, F., and Faucon, G.: Experimental Study of the Influence of Morphine on the Coronary Circulation. Cardiologia **34:** 376, 1959.

The coronary sinus outflow of the dog was measured with the heart in situ with its vascular and nervous connections left intact. Intravenously administered morphine sulfate was found not to influence the coronary blood flow, even in doses that were above the commonly accepted therapeutic range.

KARPMAN

Kozterlitz, H. W. and Taylor, D. W.: The Effect of Morphine on Vagal Inhibition of the Heart. Brit. J. Pharmacol. **14:** 209 (June), 1959.

In 4 species of animals the distal end of the severed right vagus nerve was stimulated electrically in order to slow the heart to one third of its resting rate. Intravenous morphine (0.1 to 100 mg. per Kg.) largely prevented this slowing in the rat and rabbit, had little effect in the cat, and had no effect in the guinea pig. Large doses of morphine also slowed the resting nonstimulated heart rate in the rat and rabbit. Nalorphine

reversed, at least in part, the effects of small or large doses of morphine. The possible mechanisms responsible for these effects are discussed.

ROGERS

LeMessurier, D. H., Schwartz, C. J., and Whelan, R. F.: Cardiovascular Effects of Intravenous Infusions of 5-Hydroxytryptamine in Man. Brit. J. Pharmacol. 14: 246 (June), 1959.

The effects of injecting 1, 2, or 3 mg. of 5-hydroxytryptamine solution into a forearm vein over a 10-minute period were observed 41 times in 25 presumably healthy young persons. Within 1.5 minutes after beginning the injection the pulse rate accelerated sharply, then a sustained moderate tachycardia followed. Ten to 15 seconds after the pulse rate rose, the forearm blood flow, as measured plethysmographically, increased in most subjects in a manner paralleling the pulse rate. In 2 tests the forearm flow increased similarly after brachial nerve block. 5-Hydroxytryptamine administration was followed by a variable blood pressure response, which could not be related to changes in forearm flow. Most subjects developed malar flushing and various systemic symptoms, and there was a high incidence of thrombophlebitis of the injected vein. The tachycardia was considered to result from a direct cardiac effect of the drug, and the increased forearm flow was thought to represent some secondary dilator mechanism opposing the drug's direct vasoconstrictor action.

ROGERS

McLain, P. L., Kruse, T. K., and Redick, T. F.: The Effect of Atropine on Digitoxin Bradycardia in Cats. J. Pharmacol. & Exper. Therap. 126: 76 (May), 1959.

The heart rate was studied in cats given digitoxin, 0.02 mg. per Kg., intravenously after light ether anesthesia. The heart rate was studied with and without prior administration of vagus paralyzing doses of atropine. In addition, a study was made of the effect upon sinus rates of atropine administered at a standard point during digitoxin accumulation. The results yielded no evidence of augmented vagal activity as a factor in digitoxin bradycardia. Rather, it appeared that digitoxin slowing was chiefly, if not entirely, extravagal and that in the presence of intact vagal mechanisms the effects of the drug were simply added to those of preexisting vagal tone. Atropine did not modify the rates of ectopic pacemakers produced by digitoxin. The normal pacemaker, released from vagal control, attained rates equivalent to those of ectopic pacemakers.

RINZLER

Michel, D., and Hartleb, O.: On the Question of a Potentiating Effect of Co-carboxylase on the Action of Strophanthin. Ztschr. Kreislaufforsch. 48: 557 (June), 1959.

In 14 cardiac patients over 65 years of age 100 mg. of co-carboxylase were injected intravenously 1 hour before, 1 hour after, and at the same time as ¼ mg. of strophanthin K. Co-carboxylase alone usually caused a slight decrease in the heart rate, the corrected Q-T duration, and the period of changing ventricular shape (Q-first sound), and resulted in an increase in the period of rising tension (first sound to beginning of ejection) and corrected ejection period, an increase of systolic blood pressure, and a decrease of venous pressure. These changes were more pronounced in the presence of heart failure. Strophanthin alone caused approximately similar changes with the exception of an increase of the period of changing shape and decrease of the period of rising tension. Because of the variable response statistical treatment was necessary and this showed a probable potentiation of some of the effects of strophanthin by co-carboxylase. Combination of the 2 substances seems therefore desirable, especially in patients in whom deficiency in co-carboxylase content or activity is probable because of the presence of myocardial hypoxia.

LEPESCHKIN

Millson, D. R.: Nicotine and the Effect of Antisympathomimetic Agents on the Aorta of the Rabbit. Brit. J. Pharmacol. 14: 239 (June), 1959.

Strips of rabbit aorta immersed in Ringer-Locke solution at 37 C. were stimulated to matched degrees by nicotine or norepinephrine as a test system. Of the 5 antisympathomimetic agents investigated, dibenamine, ergotamine, and tolazolamine suppressed this stimulation most strongly, although the nicotine effect was suppressed only half as strongly as that of norepinephrine. The dose-response curves for nicotine before and after phentolamine showed that the response to nicotine was readily surmountable, as would be expected if it acted by liberating a limited amount of norepinephrine within the tissues.

ROGERS

Montero, A. C., Rochelle, J. B., III, and Ford, R. V.: Clinical Pharmacology of Flumethiazide, a Diuretic Agent. Am. Heart J. 57: 484 (Apr.), 1959.

Flumethiazide was found in a clinical pharmacologic study in human subjects to be essentially similar to chlorothiazide in its diuretic action

and potency. Both drugs could be administered orally, were repeatedly effective on daily use, and were virtually free of acute toxicity. Both drugs produced similar biochemical changes, namely, a tendency toward hypochloremic alkalosis. Flumethiazide, however, appeared to have a greater effect on the excretion of water than did chlorothiazide. The data suggested that this drug might be of extreme value in the treatment of various edematous states and, perhaps, as an adjunct in the therapy of hypertension.

SAGALL

Moyer, J. H.: Human Pharmacology of Thiazide Derivatives. J.A.M.A. **170**: 2048 (Aug. 22), 1959.

Oral chlorothiazide (1 Gm.) twice daily induced excretion of the same amounts of sodium and chloride as did 2 ml. of a molar solution of mercalluride injected intravenously. After daily administration for 2 or 3 weeks, the sodium excretion declined to approach the daily intake and the patient's body weight approached a steady level about 4 pounds (1.8 Kg.) below the initial level. A dose of 2 Gm. of either chlorothiazide or flumethiazide produced a near maximal effect, and doubling the doses did not yield further significant increases in sodium excretion. For hydrochlorothiazide a dose of 200 mg. gave a maximum excretion rate. A steadier effect plus over-all increase in response resulted from giving 200 mg. every 12 hours as compared to a dose of 400 mg. once daily. Hypokalemia may follow therapy with chlorothiazide or its derivatives resulting in symptoms from hypopotassemia itself or sensitizing the myocardium to digitalis with resultant digitalis toxicity. Prophylactic administration is recommended with chlorothiazide in patients with conditions that may be associated with potassium depletion.

KITCHELL

Murnaghan, M. D.: The Antiaccelerator Action of Quinine on the Isolated Perfused Heart of the Cat. Arch. int. pharmacodyn. **120**: 312 (July), 1959.

In the isolated perfused cat's heart quinine was found to antagonize the chronotropic action of isopropylarterenol. In 7 experiments (pooled data) the mean dose required to cause a 50 per cent inhibition of acceleration (I_{50} value) was 40 μM, with the range between 22 and 66. This action is not a selective one, since quinine also decreased the amplitude and increased the coronary flow. A comparison with the results of other authors for this cinchona alkaloid showed that the heart-lung preparation of the dog is about one fifth as sensitive as the isolated heart of the cat and the isolated atria of the guinea pig, rat, and frog. On the other hand, the above-mentioned preparations of the dog, cat, rat, guinea pig, and frog display an increasing sensitivity to veratramine in this order. The results confirmed previous work that in this particular preparation coronary flow and amplitude are related.

BRACHFELD

Platis, M. M.: Hydrochlorothiazide, A New Oral Diuretic. Brit. M. J. **1**: 1565 (June 20), 1959.

Hydrochlorothiazide was studied in 3 normal patients and in 21 patients with congestive heart failure. The fact that 100 mg. of hydrochlorothiazide is equivalent in action to 1000 mg. of chlorothiazide was confirmed. The toxic effects were minimal. The action of hydrochlorothiazide was more prolonged than that of chlorothiazide. Potassium excretion by hydrochlorothiazide, and chlorothiazide was practically equivalent, hence the danger of hypokalemia with both drugs is obvious and potassium supplements should be used with each. Since hydrochlorothiazide causes urinary excretion of sodium and chloride in equimolecular quantities, the drug may produce hypochloremic alkalosis in the same way as mercurial diuretics.

KRAUSE

Poznanski, W. J. and Cromie, B. W.: Action of Chlorothiazide and "Oradon", Alone and in Combination. Brit. M. J. **1**: 1553 (June 20), 1959.

Chlorothiazide and a new oral mercurial diuretic (Oradon) were studied alone and in combination. An average of 36.6 per cent of the mercurial was recovered from the urine, indicating that it was a more potent agent than previous similar drugs. The fact that hypokalemia may develop with the use of chlorothiazide is reemphasized. This can occur even when potassium supplements are used and even when the drug has lost its diuretic effect. When Oradon was used alone, there was little derangement in electrolyte equilibrium and the drug caused very little gastrointestinal symptoms. When the 2 drugs were used together, there was potentiation of diuretic effect in every case. The control of edema in fairly severe cases could be obtained with the alternate use of the 2 drugs, giving chlorothiazide for 3 days in each week and Oradon with potassium for the other 4. Combined therapy was recommended for resistant cases, perferably in a hospital.

KRAUSE

Rand, M. and Stafford, A.: The Duration of Action of Some Cardiac Glycosides and Aglycones in the Guinea Pig. Brit. J. Pharmacol. 14: 174 (June), 1959.

The duration of action of digoxigenin, lanatoside C, oubain, digoxigenin-3-one, digitoxin, 3-acetyldigitoxigenin, digoxin, digitoxigenin, and lanatoside A—each was measured by timing the potentiation of adenosine-induced heart block in the anesthetized guinea pig by each drug. These drugs are listed in order of increasing duration of action and the latter 5, when given intravenously, acted for periods longer than 2 hours. This method is particularly suitable for testing short-acting drugs, while previous methods are better suited for testing long-acting drugs. Knowing the duration of a drug's action may provide an estimate of the rate of its elimination.

ROGERS

Stormoken, H., De Schaepdryver, A. F., and De Vleeschhouwer, G. R.: Mechanism of Bradycardia by Methoxamine. Arch. int. pharmacodyn. 120: 386 (July), 1959.

Experimental investigations in anesthetized dogs showed that the bradycardia induced by the intravenous injection of methoxamine was of reflex origin and was due primarily to a stimulation of the carotid sinus and aortic baroreceptors induced by the rise in blood pressure. The bradycardia was also related to a sensitization of the cardiac vagal responses. A direct negative chronotropic effect on the heart was also noted. Local application of methoxamine to the arterial wall of the carotid sinus areas caused a decrease of blood pressure and heart rate and a suppression of the normal carotid sinus vasopressor reflexes.

BRACHFELD

Wegria, R., Nakano, J., McGiff, J. C., Muraview, T., Zekert, H., and Blumenthal, M. R.: Effect of a New Drug, N-(2, 1)-Heptane)-Diethylaminoacetamide Hydrochloride (ERL, 239), on Mean Arterial Blood Pressure, Cardiac Output, Coronary Blood Flow, Cardiac Oxygen Consumption, Work and Efficiency. J. Pharmacol. & Exper. Therap. 126: 164 (June), 1959.

Diethylaminoacetamide hydrochloride (ERL, 239) when injected intravenously in anesthetized dogs resulted in an increase in coronary flow. This increase in coronary flow (measured as coronary sinus flow but indicating left coronary artery flow) occurred with doses that did not affect the arterial blood pressure, that lowered the arterial blood pressure slightly, and again with doses that lowered blood pressure markedly. Cardiac output and work were found to be unchanged or decreased. Cardiac oxygen consumption increased except with large doses of ERL, 239. Cardiac efficiency always was decreased.

RINZLER

PHYSICAL SIGNS

Bruns, D. L.: A General Theory of the Causes of Murmurs in the Cardiovascular System. Am. J. Med. 27: 360 (Sept.), 1959.

The commonly accepted theory that cardiovascular murmurs are the result of turbulence in flowing blood is subjected to critical analysis. On theoretical grounds, it is unlikely that turbulence in blood will generate any significant acoustical energy that could be detected as murmurs. In experiments on pulsatile flow of water and blood, at Reynolds numbers of flow well above the critical value, no murmurs were heard or recorded. It is suggested instead that cardiovascular murmurs are due to nearly periodic fluctuations in the wake downstream of any obstacle. The characteristics of this type of flow are similar to those of Aeolian tones or orifice flow. Most murmurs are due to protuberances, discontinuities of the heart, valves, or large vessels. Application of this theory explains many clinical aspects of murmurs and lends unity to a understanding of their peculiarities.

KURLAND

Mainzer, W., Pincovici, R., and Heymann, G.: Systolic Murmurs in Children. Arch. Dis. Child. 34: 131 (Apr.), 1959.

Systolic heart murmurs were discovered on routine examinations in 240 out of 2,035 children under the age of 15. A complete history was obtained from each patient who was then subjected to thorough physical, flouroscopic, electrocardiographic, and other laboratory examinations. The incidence was highest in the 5 to 10-year old group and at least 40 per cent of all murmurs occurred among siblings. Of the total number, 64.2 per cent were diagnosed as innocent and 35.8 per cent of the murmurs were considered to be definitely (3.3 per cent), possibly (19.5 per cent), or probably (13 per cent) secondary to anatomic cardiac defects. The authors hypothesize that identical anatomic construction of the ventricular outlet may be responsible for the large frequency of similar murmurs heard within the same family. They concluded that no physical criterion could be found by which to distinguish an innocent murmur, and that a complete examination should be performed in each patient with a systolic murmur.

KARPMAN

PHYSIOLOGY

Burn, J. H., and Rand, M. J.: The Cause of the Supersensitivity of Smooth Muscle to Noradrenaline after Sympathetic Degeneration. J. Physiol. **147:** 135, (June 23), 1959.

After sympathetic denervation the smooth muscles of the cat's iris and spleen were found to be supersensitive to norepinephrine. In addition, the store of norepinephrine in both organs declined and, at the same time, the sensitivity to norepinephrine increased. The authors concluded that the supersensitivity to norepinephrine and epinephrine which developed upon sympathetic denervation was due to the disappearance of a store of norepinephrine in those organs. The failure of tyramine to act on the denervated organs was attributed to the fact that tyramine acts in the normally innervated organ by releasing the stored norepinephrine and hence was ineffective in the absence of an adequate supply of norepinephrine.

KARPMAN

Holland, W. C., Klein, R. L., and Briggs, A. H.: Factors Affecting Action of Acetylcholine on Transmembrane Flux of K in Isolated Rabbit Atria. Am. J. Physiol. **196:** 478 (Mar.), 1959.

The effects of temperature, cocaine, anoxia, acetylcholine (ACh), potassium, sodium, and calcium on influx and efflux of potassium in isolated rabbit atria were studied. Increasing the potassium concentration in the medium increased both efflux and influx of potassium, especially the latter. When the concentration of potassium in the medium was 6.0 to 6.5 mM the fluxes were estimated to be equal. Acetylcholine increased both efflux and influx. In the presence of acetylcholine both fluxes are estimated to be equal at a potassium concentration in the medium of 4.0 to 4.5 mM. Studies on the influence of temperature, cocaine, and anoxia suggest that at a potassium concentration in the medium of less than 5.4 mM, the 2 fluxes were passive in nature. At concentrations above this, influx appeared to have an active component. It was suggested that at low potassium concentrations, acetylcholine affected only passive movements of potassium, whereas at higher potassium concentrations in the medium, acetylcholine stimulated active transport. A decrease of sodium in the medium to one fifth depressed efflux and influx about 50 per cent when the calcium concentration was maintained at a normal level. A decrease of calcium blocked the effect of low sodium. This suggested that calcium either increased passive movements of potassium or determined the degree of coupling between sodium and potassium fluxes.

KAYDEN

Jordan, H.: Investigations on the Relationship between Central Pulse Velocity and So-called "Delay-Time." Cardiologia **35:** 228, 1959.

Continuing previous investigations, the authors showed that the velocity of propagation of the pulse wave (determined by the method of Wezler-Boeger) has a high degree of correlation with the "delay-time" (interval between second heart sound and nadir of the incisura in the right carotid pulse tracings), and that it varies with age and blood pressure. In 36 necropsies the distance between aortic valves and the point of recording the carotid pulse was measured. Its length varied very little and did so also with age. From these distances and the "delay-time," the velocity of propagation of the pulse wave could also be calculated. Comparisons made in a variety of patients showed this to be a useful method for obtaining approximate figures of pulse wave velocity.

BRACHFELD

Quilligan, E. J., and Tyler, C.: Postural Effects on the Cardiovascular Status in Pregnancy: A Comparison of the Lateral and Supine Postures. Am. J. Obst. & Gynec. **78:** 465 (Sept.), 1959.

In a group of 196 near-term pregnant subjects, 3 per cent had a drop in systolic pressure of 30 mm. Hg or more when turned from the lateral to the supine position. The mean cardiac output, as estimated by the blood pressure method in 13 of these subjects, did not change significantly when compared in the 2 positions. The mean venous pressure measured in the legs of 5 of these subjects increased significantly. An inverse relationship between stroke volume and heart rate maintained cardiac output. Only 1 of these subjects was hypotensive to the point of syncope when turned from the lateral to the supine position.

SHEPS

Ramirez, J.: Oxidation-Reduction Changes of Cytochromes Following Stimulation of Amphibian Cardiac Muscle. J. Physiol. **147:** 14, (June 23), 1959.

The changes of the steady-state oxidation-reduction levels of the cytochromes, pyridine nucleotides and flavoproteins in the cardiac muscle of the frog and toad were followed during and after isometric contractions by means of spectrophotometric technics. The components of the respiratory chain in the intact cardiac muscle were similar to those in the toad heart sarcosomes and 2 to 3 times higher than in the toad's sartorius muscle. During mechanical activity the maximum change of the respiratory chain

components was very small (2 to 6 per cent) and occurred quite rapidly. The pyridine nucleotides, flavoprotein, and cytochromes b and a_3 were oxidized during activity and cytochromes c and a were usually reduced. The changes of the respiratory chain components in the sarcosomes on addition of ADP were the same as found in contracting muscle. Cytochrome a_3 did not change upon addition of ADP to the sarcosomes. The author concluded that the respiratory chain is the main source of ATP resynthesis in the heart.

KARPMAN

Sabiston, D. C., Jr., Talbert, J. L., Riley, L. H., Jr., and Blalock, A.: Maintenance of the Heart by Perfusion of the Coronary Circulation with Gaseous Oxygen. Ann. Surg. **150**: 361 (Sept.), 1959.

The experiments in this paper were designed to evaluate the effect of perfusion of the dog heart with gaseous oxygen. These observations were made both on the isolated heart and the heart remaining intact within the chest. It has been shown that, under experimental conditions, oxygen may be provided to the tissues by electrolyte solutions highly saturated with oxygen and perfused into the vascular system. Proportionately the heart utilized oxygen to a much greater extent than the rest of the body. These experiments indicated that the heart beat in the isolated heart may be maintained up to 8 hours with perfusion of the coronary circulation with gaseous oxygen. With the heart in situ the same type of oxygen perfusion has been performed with later reversion to a normal circulation. Gaseous embolism did not occur under these circumstances. It was noted that the empty heart requires about 38 per cent of the oxygen necessary for the normal beating heart and that a heart, even in asystole, requires appreciable amounts of oxygen. A mixture of 95 per cent oxygen and 5 per cent carbon dioxide was used for the perfusion of the dog hearts employed in these experiments. In the hearts in situ little difference was noted between the use of this mixture, 100 per cent oxygen, or compressed air. In the experiments in situ, although circulation was restored, most of the animals never regained consciousness. It was believed that this was due to a prolonged period of anoxia for the remainder of the body and suboptimal cerebral perfusion. The studies would indicate merely that adequate cardiac function may be maintained for several hours with only gaseous oxygen perfusion of the coronary circulation.

LEVINSON

Scott, J. C., and Balourdas, T. A.: An Analysis of Coronary Flow and Related Factors Following Vagotomy, Atropine and Sympathectomy. Circulation Research **7**: 162 (Mar.), 1959.

Coronary blood flow was measured in the dog by the nitrous oxide desaturation method. The removal of cardiac vagal control by cervical vagotomy or administration of atropine resulted in a markedly increased coronary flow, myocardial oxygen consumption, and heart rate, but a decreased left ventricular efficiency. Sympathectomy resulted in changes in the opposite direction. The effects of other variables, such as type of anesthesia and oxygen content of the nitrous oxide-oxygen mixture on these findings are discussed.

PAUL

PULMONARY DISEASES

Franco Browder, S., Perez Olea, J. O., and de Francisco, A.: Pulmonary Infarction in the Cardiac Patient. Arch. Inst. cardiol. México **29**: 31 (Jan.-Feb.), 1959.

The authors noted a high incidence of pulmonary infarction in patients with heart disease. This complication occurred in the most varied types of heart disease and in this series was found particularly in myocardial infarction with fatal outcome. The pathologic diagnosis of pulmonary infarct permitted the analysis of some clinical, radiologic, and electrocardiographic elements that the authors consider useful for the identification of the clinical picture of pulmonary infarction. The most outstanding complications were aggravation or the enhancement of heart failure, bouts of acute pulmonary edema, and digitalis intoxication.

BRACHFELD

Griffin, J. T., Kass, I., and Hoffman, M. S.: Cor Pulmonale Associated with Symptoms and Signs of Asthma in Children. Pediatrics **24**: 54 (July), 1959.

Three case reports are presented of children manifesting pulmonary hypertension and right ventricular hypertrophy associated with signs and symptoms of asthma. The authors emphasize that cor pulmonale may be a complication of asthma in children as well as in adults. They suggest that cardiopulmonary function studies be performed on all children with bronchial asthma, especially when the asthma is responding poorly to treatment or when evidence of decreased pulmonary function is detected.

KARPMAN

NEWS FROM THE AMERICAN HEART ASSOCIATION

44 East 23rd Street, New York 10, N.Y.

Telephone Gramercy 7-9170

Program of Wide Interest Planned For AHA Scientific Sessions

Because of their enthusiastic acceptance last year, six sessions of broad clinical interest sponsored by the AHA Council on Clinical Cardiology are being planned for the American Heart Association's 33rd annual Scientific Sessions to be held from Friday, October 21 through Sunday, October 23 at Kiel Auditorium in St. Louis. The clinical sessions will run concurrently with special scientific sessions and programs sponsored by other Councils of the Association.

Following a similar program presented successfully last year, the tentative program also includes a session on medical motion pictures to be introduced by the author or other authority on the subject.

Abstracts of Papers Due June 1 For AHA Scientific Sessions

June 1 is the deadline for submitting *abstracts of papers* to be presented at the 33rd annual Scientific Sessions of the American Heart Association, scheduled to be held October 21-23 in St. Louis. Papers intended for presentation must be based on original investigative work in the cardiovascular or related fields. Abstracts must be limited to 250 words or less and include a brief digest of results obtained and conclusions reached. The AHA Committee on Scientific Sessions Program will screen all applications received.

Official forms for submitting abstracts of papers and applications for space for *scientific exhibits*, which also must be returned by June 1, may be obtained from F. J. Lewy, M.D., Assistant Medical Director of the American Heart Association, 44 East 23rd Street, New York 10, N. Y.

Applications for space for *industrial exhibits* may be obtained from Steven K. Herlitz, Inc., 280 Madison Avenue, New York 16, N. Y.

Arteriosclerosis Council Meets October 19-21, Abstracts Due June 1

June 1, 1960 is the deadline for submitting abstracts of papers to be presented at the meeting of the Association's Council on Arteriosclerosis in the Sheraton-Jefferson Hotel, St. Louis, Wednesday through Friday, October 19-21. Factual abstracts of the material to be presented, not exceeding 250 words, consisting of the original and four copies should be sent to Campbell Moses, M.D., Program Chairman, University of Pittsburgh School of Medicine, Pittsburgh 13, Pa.

The Council will hold its 14th independent scientific meeting Wednesday and Thursday, October 19 and 20 immediately preceding the Annual Meeting and Scientific Sessions of the American Heart Association. The Council will participate in a scientific session of the American Heart Association on Friday, October 21.

All sessions of the Council will be open to all interested individuals who are invited to submit abstracts of papers for presentation regardless of membership in the Council.

Montana Heart Group Donates $5,000 To Supplement National Research

The Montana Heart Association has contributed $5,000 to supplement the Association's national research support program for the

1960-61 fiscal year. This, and other sums to be donated by Heart Associations in addition to amounts regularly assigned by them for national research, will permit support of additional investigations beginning July 1, 1960 which could not be covered by the national research budget.

Affiliates and chapters of the Association contributed a total of $68,680 to help reduce a deficit in the national research budget and to aid in underwriting a record total of $3,300,000 awarded for national research in the 1959-60 fiscal period.

Meetings Calendar

May 16-20: Postgraduate Course, "Current Research in Cardiovascular Disease," directed by Dr. Luther L. Terry, Bethesda, Md. National Heart Institute, Bethesda 14, Md.

May 23-27: Postgraduate Course, "The Hypertensive Diseases: Diagnostic and Therapeutic Procedures in Eessential, Adrenal and Renal Hypertension," directed by Dr. Robert W. Wilkins, Boston. Boston University School of Medicine, Boston, Mass.

May 23-28: American College of Cardiology, Indianapolis. Philip Reichert, 2709 Empire State Bldg., New York 1, N. Y.

June 8-12: American College of Chest Physicians, Miami Beach. Murray Kornfeld, 112 E. Chestnut St., Chicago 11, Ill.

June 11: International Cardiovascular Society, North American Chapter, Miami Beach. P. T. DeCamp, 3503 Prytania St., New Orleans 15, La.

June 12: Society for Vascular Surgery, Miami Beach. G. H. Yeager, 314 Medical Arts Bldg., Baltimore 1, Md.

June 13-17: American Medical Association, Annual Meeting, Miami Beach. F. J. L. Blasingame, 535 N. Dearborn, Chicago 10, Ill.

August 8-11: National Medical Association, Pittsburgh. John T. Givens 1108 Church Street, Norfolk, Va.

August 21-26: International Congress of Physical Medicine, Washington, D.C. W. J. Zeiter, 2020 E. 93rd St., Cleveland, Ohio.

August 28-September 3: International Society for Welfare of Cripples, 8th World Congress, New York. Donald V. Wilson, 701 First Ave., New York 17, N. Y.

September 11-15: International College of Surgeons, New York. Max Thorek, 850 W. Irving Park Road, Chicago, Ill.

September 18-21: International Meeting of Forensic Pathology, New York. Milton Helpern, 55 East End Ave., New York 28, N. Y.

September 27-30: American Roentgen Ray Society, Atlantic City. Hugh Jones, 20 N. Wacker Drive, Chicago 6, Ill.

October 10-14: American College of Surgeons, Clinical Congress, San Francisco. W. E. Adams, 40 E. Erie St., Chicago 11, Ill.

October 21-25: American Heart Association, Annual Meeting and Scientific Sessions (October 21-23), St. Louis. American Heart Association, 44 East 23rd St., New York 10, N. Y.

October 31-November 2: Association of American Medical Colleges, Hollywood Beach, Fla. Ward Darley, 2530 Ridge Ave., Evanston, Ill.

October 31-November 4: American Public Health Association, San Francisco. B. F. Mattison, 1790 Broadway, New York 19, N. Y.

November 28-December 2: American Medical Association, Clinical Meeting, Washington, D. C. F. J. L. Blasingame, 535 N. Dearborn, Chicago 10, Ill.

November 30-December 3: Canadian Heart Association and National Heart Foundation of Canada, Toronto. J. B. Armstrong, 501 Yonge Street, Toronto 5, Canada.

Abroad

May 30-June 3: Asian-Pacific Congress of Cardiology, Melbourne, Australia. A. E. Doyle, Alfred Hospital, Melbourne, S. 1, Victoria, Australia.

June 2-4: International Symposium on Drugs Affecting Lipid Metabolism, Milan, Italy. Prof. S. Garattini, Institute of Pharmacology, Via A. Del Sarto 21, Milan, Italy.

August 14-20: Inter-American Congress of Cardiology, Rio de Janeiro. Magalhaes Gomes, Av. Nilo Pecanha, 38, Rio de Janeiro, Brazil.

August 24-27: International Congress of Internal Medicine, Basel, Switzerland. Secretariat, 13 Steinentorstre, Basel, Switzerland.

August 28-September 1: International Congress on Diseases of the Chest, Vienna. Murray Kornfeld, American College of Chest Physicians, 112 E. Chestnut Street, Chicago 11, Ill.

September 1-4: First International Congress of Nephrology, Geneva and Evian, France. G. Richet, 149 Rue de Sevres, Paris 15, France.

September 4-10: International Society of Hematology, Tokyo. J. L. Tullis, 1180 Beacon Street, Brookline 46, Mass.

September 18-25: European Congress of Cardiology, Rome. V. Puddu, Clinica Medica, University of Rome, Italy.

1962:—October: Fourth World Congress of Cardiology, Mexico City. I. Chavez, Ave. Cuauhtemoc 300, Mexico, D. F.

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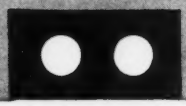
Just two tablets  *at bedtime*

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Circulation

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OFFICIAL JOURNAL of the AMERICAN HEART ASSOCIATION

SYMPOSIUM on SALT and WATER METABOLISM

NEW YORK HEART ASSOCIATION

New York City, December 11-12, 1959

Alfred P. Fishman, M.D.
Guest Editor

Published by the American Heart Association, Inc.

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CIRCULATION

AN OFFICIAL JOURNAL OF THE AMERICAN HEART ASSOCIATION

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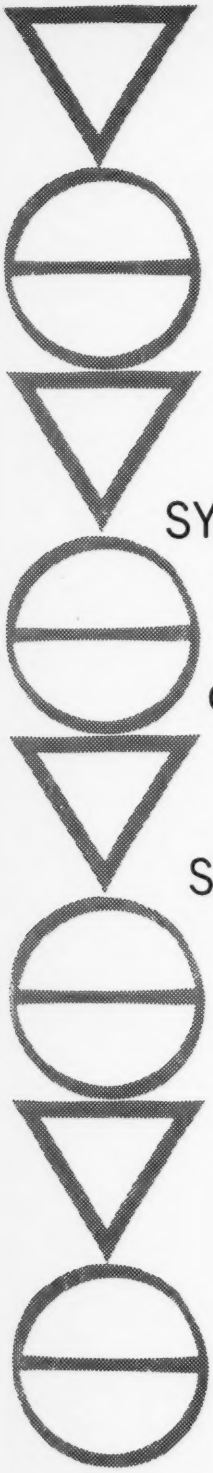

SYMPOSIUM

on

SALT and WATER METABOLISM

NEW YORK HEART ASSOCIATION

New York City, December 11-12, 1959

Alfred P. Fishman, M.D.

Guest Editor

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VOL. XXI NO. 5
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AN OFFICIAL JOURNAL of the AMERICAN HEART ASSOCIATION

Foreword

THIS volume is a record of the papers which were presented at a symposium on salt and water metabolism, and of the discussions which they evoked. The meeting was sponsored by the New York Heart Association and was held on December 11-12, 1959. The participants shared a lively interest in salt and water metabolism but differed considerably in their scientific orientations: they included physical chemists, physiologists, zoologists, and physicians; their topics ranged from the physical chemistry of solutions to the transport of electrolytes and water across biologic membranes.

The record has been slightly modified for publication. However, the changes have been editorial rather than conceptual or factual. The actual proceedings have also been supplemented in 2 ways: (1) by the inclusion of a readable abstract before all but the introductory papers; and (2) by the use, as space fillers, of pertinent quotations from other publications.

The general plan of the meetings is indicated on the preceding pages. The first session, under the title of "Theory of Solutions," dealt with fundamentals, i.e., the nature of water, of water adulterated by electrolytes and proteins, and of biologic membranes. To the extent that these papers represent common opinion, they indicate that the physical chemist has more respect for biologic solutions and membranes than does the biologist. They also suggest how oversimplified may be the concepts of biologists who consider renal transport mechanisms in terms of simple solutions, inert substances, and cellophane-like membranes.

At first glance, the second session may seem to have reverted to familiar biologic grounds, i.e., the role of the kidney in the regulation of salt and water metabolism. But this ordinary topic conceals an unusual emphasis; instead of dealing with entire kidneys, the meeting dealt primarily with individual nephrons. New techniques were considered in their role of substantiating old concepts and in suggesting new ones. Parallels were drawn between the exchange of ions and water across renal tubular cells and across biologic membranes elsewhere. The collecting ducts were shown to participate in the regulation of the final concentration of solutes in the urine.

On the grounds that many extrarenal tissues and organs are involved in the exchange of ions and water, the second day was devoted to the *extrarenal* regulation of salt and water metabolism. Two particular aspects were chosen for consideration: (1) the salt and water exchange of extrarenal organs and tissues; and (2) the extrarenal control of excretion of salt and water by the kidney. The designation of a 2-day limit for the symposium automatically limited the number of topics which could be considered. The reader is referred elsewhere[1-4] for discussions of ion transport across red cells, the frog skin, the cerebrospinal membranes, nerve and muscle, and for some of the practical implications of these exchanges.[5]

Throughout the entire meeting ran the threads of comparative anatomy, physiology

and biochemistry. The specialized information which comparative biology can provide was evident in the micropuncture studies of amphibian and mammalian kidneys, the chemical analyses of the secretion of the "salt gland" of marine birds, the comparisons of the biochemical composition and the activities of the different antidiuretic hormones; broader perspectives were evident in the consideration of salt and water metabolism and its relationship to vertebrate metamorphosis.

The meeting appeared to excite the interest and imagination of the participants and of the audience. Unfortunately, a transcript such as this is hard put to reproduce either the liveliness of the proceedings or the vital personalities of the participants; it may, however, provide for the reader—through the trained and critical eyes of the participants—a fresh insight into our present understanding of salt and water metabolism.

ALFRED P. FISHMAN
Guest Editor

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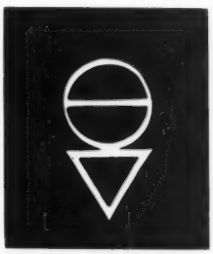

Salt
Water

Koch, R. *The Book of Signs.* Translated from the German by V. Holland. London, First Editions Club, 1930, pp. 49, 66.

I. THEORY OF SOLUTIONS

CHAIRMAN: HOMER W. SMITH, SC.D.

"A Knowledge of the Laws of Solutions . . ."

By HOMER W. SMITH, SC.D.

"A KNOWLEDGE of the laws of solutions," it has been said, "is important because almost all the chemical processes which occur in nature, whether in animal or vegetable organisms, or in the nonliving surface of the earth, and also those which are carried out in the laboratory, take place between substances in solution. For example, a sound judgment regarding physiological processes . . . is impossible without this knowledge; and this holds true for the greater number of the scientifically and technically important reactions. . . . Solutions are more important than gases, for the latter seldom react together at ordinary temperatures, whereas solutions present the best conditions for the occurrence of all chemical processes.

"The discovery of the laws of solutions is full of significance for the advance of physical chemistry. . . . The colligative laws which apply to gases and dilute solutions always maintain their character because the molecules of gases and of [very] dilute solutions are so far removed from one another that neither their mutual interaction nor their special nature, but only their numbers, come into play. But the individual characters of the molecules become more important when gases are compressed, or solutions are concentrated, and deviations from the colligative laws dominate more and more, until at last, in pure liquids produced by the continued compression of gases below their critical temperatures, and in the solid state, produced by the removal of all solvent, [intermolecular forces completely dominate].

"The hope may be expressed that the possibility of representing and studying all these intermediate states, which can be accomplished easily and fully in the examination of solutions, will be of considerable help in making easier the study of the laws of pure liquids." The foregoing is quoted, with slight paraphrase, from the second edition of Wilhelm Ostwald's *Lehrbuch der allgemeinen Chemie* of 1891.[1*]

Ostwald's generalization stemmed largely from 2 papers, then only a few years old, one by the Dutchman, Jacobus Hendricus van't Hoff (1852-1911), the other by the Swede, Svante August Arrhenius (1859-1927), which contributed the major foundations of our contemporary physical chemistry, of some of our contemporary physiology, and, in no small measure, of that sprightly if as yet ill-defined contemporary discipline, the proponents of

From the Department of Physiology, New York University College of Medicine, New York, N. Y.

*Ostwald's *Lehrbuch* had a rather checkered history. The first edition in 2 volumes was published at Riga in 1885-87, but rapid progress in physical chemistry led to a second edition of 2 volumes, published at Leipzig in 1891-93. A revised, second printing of this second edition was published at Leipzig in 1896-1903,[4] with the second volume in 2 parts, followed by a fragment of a third part. The first printing of the second edition is not available to the writer, and he has followed the second printing. Vol. 1, Book 4 (*Solution*) of the first printing has been translated by Muir.[1]

which choose to call themselves "biophysicists." Current studies of the nature of solutions are still in the stage of revising and improving the pioneer ideas of van't Hoff and Arrhenius.

• • •

Van't Hoff had thought to be a chemist, but as an undergraduate student at the University of Leyden he yielded to the lure of mathematics, a subject wholly foreign to the prosaic synthetic-analytic chemistry of the 1870's. In Bonn he worked with Friedrich Kekulé, discoverer of the tetravalent nature of carbon and the ring structure of benzene; and in Paris with Charles Würtz, who proudly said that chemistry was "a French science"—without fulminating either himself or his mentors. From Paris he went to Utrecht where, in 1874, he won his Ph.D. with a conventional and wholly safe thesis on cyanacetic and malonic acids,[2] wisely refraining from drawing the attention of his doctoral examiners to an heretical 11-page pamphlet which, shortly before his examination, he had published privately. The Dutch title of this pamphlet is beyond my lingual ability, and even in English it is too long to read.[3*] Twenty-four years elapsed before this pamphlet reached the English language, whereupon the title was reduced to the simple phrase, *The Arrangement of Atoms in Space* (1898).[6] Thereafter this work won for van't Hoff an international reputation as the founder of stereochemistry, which deals with the arrangements of atoms and molecules in 3 dimensions instead of 2. Through the instigation of Johannes Wislicenus the pamphlet was translated into German in 1877,[7] whereupon it was denounced by Hermann Kolbe of Leipzig, one of the most eminent organic chemists of the time but one who could not think in 3 dimensions, as "fanciful nonsense [which] carefully avoids any basis of fact, and is quite unintelligible to the calm investigator. . . ." As for Wislicenus, Kolbe said that "he has gone over from the camp of the true investigators to that of the speculative philosophers of ominous memory, who are separated by only a thin medium from spiritualism." [8, pp. 86-87]

Van't Hoff's capacity for abstraction had early led to an interest in chemical kinetics, and his thoughts now leaped from 3 to 4 dimensions. "Good music," he once said, "makes it very pleasant to think of other things"—other things, perhaps, being his second great work, his *Études de Dynamique Chimique* of 1884.[9] This work was translated into German[10] and English[11] 12 years after its publication in French; it is frequently mentioned but rarely read because in any language it is very scarce. In the *Études* van't Hoff incorporated all that had hitherto been known and added so much that was new that, so it has been said, chemical dynamics was scarcely improved in the next 30 years.[8, p. 343] Though the Table of Contents of the *Études* suggests a veritable encyclopedia (which in a sense it is), van't Hoff's writing is marked by brevity and clarity, and by careful definition of all mathematical terms; where a mathematical relation represents a broad generalization, this generalization is re-expressed in words. He did not concur in the view of Willard Gibbs, who once asserted that "mathematics is a language" and who generally chose to speak no other.*

The *Études* did not evoke the ridicule that had greeted *Atoms in Space*; rather it was received in silence because there seemed to be no one who could comprehend its dynamic approach—until in a Norwegian review[12] for March, 1885, there appeared an exhaustive critique giving it its proper valuation as one

*The Dutch pamphlet was translated by van't Hoff into French in 1875,[5] a translation made necessary by the fact that Joseph Achille Le Bel (1847-1930) of Paris had expressed very similar views in November of 1874. Le Bel and van't Hoff had come to the theory of the asymmetry of the carbon atom independently, but because of van't Hoff's priority and more thorough treatment of the problem of optical activity he is generally given the major credit.

*"It is said that, during his long membership in the Yale faculty, Willard Gibbs made but one speech, and that of the shortest. After a prolonged discussion of the relative merits of language and mathematics as elementary disciplines, he rose to remark, 'Mathematics is a language.' "[15, p. 26]

of the classics of science. This critique was written by a relatively unknown chemist at the University of Upsala, one who was destined shortly to become as famous as van't Hoff himself, Svante August Arrhenius.

It was in the last section of the *Études,* which deals with the topic of chemical affinity, that van't Hoff first propounded his gaseous theory of solution. He had been thinking to determine the affinity of Glauber's salt ($Na_2SO_4 \cdot 10H_2O$) for its water of crystallization by measuring the vapor pressure of water in equilibrium with the dry solid. Then it came about that one day in 1883, probably just before the *Études* went to the printer, on leaving his laboratory in Amsterdam van't Hoff and his wife met the botanist Hugo de Vries (1848-1935) (of "mutation" fame, and who was Professor of Botany at the University), and de Vries talked to him of the plasmolysis of plant cells, of isotonic solutions of sucrose and salts, and of the remarkable quantitative studies in osmotic pressure carried out by the botanist Wilhelm Friedrich Philip Pfeffer (1845-1920) and summarized in 1877 in Pfeffer's monumental monograph, *Osmotische Untersuchungen.*[13]

* * *

The discovery of osmotic phenomena had been made in 1748 by the Abbé Jean-Antoine Nollet[14] who, in seeking the source of the bubbles that form in liquids when they are boiled, had covered a vial of boiled spirits with a membrane—presumably of pig's bladder—and submerged the covered vial under water. He noticed that in a short time water moved into the vial, causing the membrane to bulge outward with great pressure. The paradox, that water should move into a sealed vial against pressure, he correctly explained, after numerous well-controlled experiments, by the supposition that the membrane is more permeable to water than to alcohol. (The Abbé's paper was published half a century before Dalton (1803) formulated the atomic theory, a full century before Cannizzaro (1858) resolved the long controversy between those who went along with Avogadro and those who went along with Berthollet.)

There followed a century and more of unobtrusive experimentation on osmosis, beginning with Parrot, who in 1815 studied the mixing of liquids by diffusion. Some 15 years later the physiologist Dutrochet introduced the first, if crude, manometer into this problem and in 1827 Dutrochet[16] coined the words "endosmose" and "exosmose," meaning to push or impel. He later showed that osmosis can occur through a thin sheet of marble, and that organic membranes are generally slightly permeable to salts. The history of our problem in the next few decades bears many familiar names: Fischer, Gustav Magnus, Jerichau, Brücke, Matteucci and Cima, Liebig, Jolly, Vierordt, Eckard, and—not the least among physiologists—Carl Ludwig, who based his filtration-reabsorption theory of renal function on his own osmotic experiments and those of others. Important among chemists was Thomas Graham, who in 1826 formulated the basic laws relating the diffusion of gases to their density, applied these laws to the diffusion of a solute through membranes in 1854, and in 1861 divided solutes into crystalloids and colloids. It was Graham who translated Dutrochet's *osmose* into the English "osmotic."* The generalized law of diffusion, formulated in 1855 by A. Fick, is so well known as to require its mention only.

Interest in osmosis spread from physiology to botany when Pringsheim in 1854 and Naegeli in 1855 showed that the protoplasmic contents of plant cells contract in strong sugar or salt solutions, indicating that the surface of the protoplasm constitutes a membrane permeable to water but not to many solutes. Moritz Traube is hard to classify: a pupil of Liebig's, he was primarily engaged in the wine business, but worked on chemical and biological problems, fermentation, and cell theory on the side. He had discovered in 1867 that osmosis could be studied by means of membranes precipitated from colloidal or other solutions over the open ends of glass tubes; and, seeking a better membrane, he had

*J. Hogg used the nominative form "osmosis" in 1867, but the verbal "osmose" remained in use until the end of the century.

ome upon the film of copper ferrocyanide. Pfeffer, who, like Pringsheim and Naegeli, had been studying plasmolysis (and who seemed to have been the first to have spoken of the bounding surface of the plant cell as the "plasma membrane"), saw the potentialities of Traube's artificial membrane and in 1877 reported the most important single advance in the study of osmosis: he had precipitated the copper ferrocyanide film in the porous wall of an unglazed earthen pot, thus preparing the first truly rigid osmometer by which osmotic pressure could be measured accurately at widely different concentrations and temperatures. It was about this date that Hamburger began his studies on the hemolysis of red blood cells, and that de Vries himself had begun his studies using (as he called it) the "plasmolytic" method. In the ensuing 8 years de Vries had established the "isotonic" concentrations (another word of his) for numerous electrolytes and nonelectrolytes, using a variety of plant cells.

* * *

Hence, it seems that the stars of science were in some sort of favorable conjunction over Amsterdam when de Vries, the isotonically minded botanist who was well informed on Pfeffer's plasma membrane and copper ferrocyanide manometer, met van't Hoff, the disillusioned organic chemist who thought in terms of 4 dimensions and whose *Études* had practically given birth to the science of chemical dynamics.

And so it was that in the last, hurriedly written chapter of the *Études* of 1884, van't Hoff's attention turns from the chemical affinity of Glauber's salt for its water of crystallization, to the attraction which an aqueous solution has for pure water, as revealed by either de Vries' plasmolytic method or Pfeffer's osmometer. Here van't Hoff coins the word "semipermeable" to describe Pfeffer's copper-ferrocyanided pot because it permits the passage of water but not of sucrose. Our erstwhile chemist proceeds with prescient inspiration to show that the osmotic pressure of Pfeffer's solutions is related to the (natural logarithm of the) ratio of the vapor pressure of pure water divided by the vapor pressure of the solution; and to this end he calculates the needed vapor pressures from the relation, established in 1870 by Guldberg (of mass-law fame), between freezing-point lowering and vapor-pressure reduction,[17] using data on the freezing point of sucrose solutions published by Raoult in 1883.[18]

Then, it seems as though haste to get the manuscript of the *Études* off to the printer lands him in an anticlimax: using the recorded vapor pressure data, he calculates that if 2 molecules of water are removed from $CuSO_4 \cdot 5H_2O$, an osmotic pressure of 1,300 atmospheres would be produced. He adds, "The experiment could not of course be carried out in the usual way [i.e., in Pfeffer's pot] since we are here dealing with a solid substance."

* * *

That is all. Van't Hoff had the rational approach to the study of solutions in his *Études* of 1884, and he abandoned it. Within the year he had turned one of those narrow corners that make of life a tangled skein. On October 14, 1885, he purportedly "read" a "paper" in Stockholm before the Royal Swedish Academy of Sciences, entitled "The Law of Chemical Equilibrium in the Dilute State, Gaseous or Dissolved." This paper was published in 3 parts in the *Transactions* of the Academy of 1886.[19*]

Now, there is one thing of which I am certain, and that is that van't Hoff never "read" this paper anywhere. As published, it runs to some 57 large pages replete with tabular data, diagrams, and differential equations. He undoubtedly said one thing and published another—but this is no sin because we all have committed it. The spoken and written word are different dialects within the mother tongue. What he did say to the academicians we will probably never know.

In the printed paper, however, he draws the analogy between de Vries' plant cells and Pfeffer's copper ferrocyanide osmometer; and, under the principle of conservation of energy

*Part I of this paper was republished, with minor changes, in Holland in 1886.[20]

and the Carnot-Clausius principle (that heat does not pass spontaneously from one body to another one which has a higher temperature), he applies a reversible piston-compression cycle to 2 ideal fluids separated by an ideal semipermeable membrane. Using Pfeffer's data on the osmotic pressure of sucrose at various concentrations and temperatures, de Vries' data on the isotonic concentrations of various solutes, and Hamburger's data on the hemolysis of red blood cells, he deduces the relations between 3 of the 4 colligative properties of solutions: osmotic pressure, freezing-point lowering, and vapor-pressure reduction.* And he shows that, for very dilute or "ideal" solutions in which intermolecular forces can be neglected the osmotic pressure conforms with the laws describing dilute or ideal gases, in the senses that:

1. As stated by Boyle for gases, osmotic pressure is proportional to the concentration of solute, the temperature remaining constant.

2. As stated by Gay-Lussac for gases, osmotic pressure is proportional to the absolute temperature, the concentration remaining constant.

3. As stated by Avogadro for gases, other conditions remaining constant, osmotic pressure is the same for any 2 solutions containing the same number of solute molecules per unit volume; and furthermore, this pressure amounts to 22.4 atmospheres at 0° Celsius,* the figure 22.4 being all the more remarkable when one considers the difference between the gram-molecular weight of gaseous hydrogen, which is 2, and that of dissolved sucrose, which is 342.

And so he applies to dilute or ideal solutions the familiar ideal gas law equation:

$$PV = nRT$$

where P is now osmotic pressure instead of gas pressure and n is the number of mols† dissolved in 1 liter of solution.

In short, he says that the osmotic pressure of a dilute solution is equal to the pressure which the solute would exert if dispersed as a gas at the same temperature and in a volume equal to that of the solution.

In van't Hoff's interpretation, osmotic pressure depends on the impact of the molecules of the solute against the semipermeable membrane—for the all too obvious and equally erroneous reason that "the molecules of the solvent, being present upon both sides of the membrane through which they pass [freely!],

---

*Guldberg, we have noted, had deduced the relation between freezing-point lowering and vapor-pressure reduction in 1870,[17] a relation experimentally confirmed by Raoult in 1878.[21] Van't Hoff now had available to him Raoult's demonstration that: (1) the reduction of freezing point of equimolecular aqueous solutions is independent of the nature of the solute;[18] and (2) the reduction of freezing point at equimolecular concentrations in various solvents is constant (water being a notable exception).[22]

The 3 interrelated properties of a solution—osmotic pressure, freezing-point lowering, and vapor-pressure reduction—were, on the suggestion of Wundts, called "colligative" by Ostwald in 1891.[1, p. 30] Ostwald distinguished additive, constitutive, and colligative properties: additive properties are those which are the sums of the properties of the constituents (e.g., mass); constitutive properties depend on the arrangement of the constituents of a pure substance (e.g., boiling point, optical activity, etc.); colligative properties are those which have equal values for chemically comparable (molar) quantities of the most different substances (e.g., volume and pressure relations of gases, and the aforementioned properties of solutions in which the component molecules are so widely separated from one another that the mutual interactions of these molecules are reduced to a minimum).

It was not until 1889 that Beckmann[23] added boiling-point elevation to the other 3 colligative properties, the theoretical relationships having been supplied by "my honored friend" Arrhenius.

*He means centigrade, of course, because Celsius' thermometer was the centigrade thermometer turned upside down.

†n has here been added to van't Hoff's gas law equation simply to round out his thought. The "kilogram equivalent" was apparently introduced into the gas law equation by Horstmann in 1881,[25] and convenience led rapidly to the substitution of the "gram equivalent" or "gram molecule." The latter was first called a *Mole* by Ostwald in 1902,[4, printing 2, p. 612] when this writer also coined the phrase *Molenbruch*, or *mol fraction*, to describe the molecular mixture formula, $\frac{n_2}{n_1 + n_2}$, first used by Raoult in 1888 in his general law of vapor pressure reduction of ethereal solutions:[26, 27]

$$\frac{p - p'}{p} = \frac{n_2}{n_1 + n_2}.$$

to not enter into consideration.''*[24, p. 15†] He recognized, however, that one could equally well treat osmotic pressure in terms of the attraction of the solute molecules for the solvent molecules—in dilute solutions the bombardment and attraction theories would be mathematically indistinguishable. The third interpretation, that osmotic pressure reflects the difference in vapor pressure of the solvent in the pure state and in the solution, that this vapor-pressure difference is indeed the *vis a tergo* of osmosis, was germinal in the *Études* of 1884; but he had turned the narrow corner before he went to Stockholm and thenceforth he follows the wrong road.

Why?

The obvious fact of the 2-way traffic of the solvent through the membrane can scarcely by itself explain van't Hoff's predilection for the ''bombardment'' interpretation because in the *Études* he had clearly recognized that the great affinity which $Na_2SO_4 \cdot 10H_2O$ exhibits for its water of crystallization is reflected in a proportional reduction in the vapor pressure of water above the salt; and in discussing Pfeffer's experiments he had utilized a reversible cycle consisting of a semipermeable membrane *and* a vapor phase—''The pressure,'' he said, ''which causes the flow of vapour from [one chamber to the other] is therefore equal to the diminution in the vapour pressure of water which is produced by dissolving the salt in it; . . .'' Hence one may suspect that he was also influenced by that charm which is only skin deep (and by which too many of us have at times been swayed)—namely, the esthetic appeal of his theory. The gaseous theory of solution, the miraculous figure of 22.4, promised the answer to an age-old mystery of why sugar dissolves in water, an answer both beautiful and simple—it turns into a gas. But nature is neither beautiful nor simple; it is damnably puzzling and complex, and no one yet has turned sucrose into a gas or even explained a semipermeable membrane.

* * *

However, to return to matters of record, from the beginning van't Hoff recognized that only dilute solutions conform with the gas law equation, and then, *only* dilute solutions of a few substances—sucrose is the classical example. *Most* substances, such as strong acids, strong bases, and most salts, which Faraday long ago had called electrolytes* because their aqueous solutions conduct the voltaic current, behaved anomalously not only with respect to osmotic pressure but also with respect to vapor-pressure lowering and freezing-point reduction. For this anomalous behavior he had no explanation.

The Stockholm paper of 1885 was reworked in 1887 for publication in volume 1 of the new *Zeitschrift für physikalische Chemie*, of which Ostwald and van't Hoff were co-editors. (Nothing clarifies our ideas so much as rewriting a paper once a year, perhaps for several years.) Van't Hoff has now seen Raoult's paper on ''The General Law of the Vapor Pressure of Solutions,''[29] showing that for equimolecular concentrations of nonvolatile

*Ostwald echoes this argument in 1981 when he writes ''There is no doubt that the cause of [osmotic] pressure is to be sought for in the dissolved substance, for water cannot produce any enduring pressure, inasmuch as it passes through the separating membrane without difficulty.'' How close he came to the proper interpretation is revealed by the later comment that ''the [osmotic] cell behaves as if there were a partial vacuum for water in its interior; water flows in, and, if no opposing pressure is allowed to develop, produces a continuous movement which ceases only when the contents of the cell have become the same as those of the space surrounding the cell, i.e., have become pure water. A condition of [osmotic] equilibrium is possible only when the pressure which prevails within differs sufficiently from that which prevails without.''[21, pp. 101-102] The belief that only the solute (not the solvent) in the osmometer is under pressure was expressed as late as 1928 by Bancroft and Davis.[28]

†This volume contains an excerpt of reference[13] and all of references[22, 26, 27, 29, 32 and 37] translated into English by Jones. Reference[37] is reproduced in part in Leicester, H. M. and Klickstein, H. S.: *A Source Book of Chemistry: 1400-1900.* New York, Toronto and London, McGraw-Hill Book Co., Inc., 1952, pp. 489-490.

*Faraday had introduced the words ''electrode,'' ''electrolyte,'' ''electrolysis,'' and ''ion'' in 1839. ''Ion'' he derived from the Greek *ion* meaning traveling or traveler.

solutes the vapor pressure of various solvents is reduced by the same fraction: i.e, solutions that have the same osmotic pressure have the same vapor pressure—but for van't Hoff, vapor pressure still remains merely another of the "colligative" properties of a solution.

* * *

Something new, however, is added in the *Zeitschrift* paper, namely, an explanation of the anomalous behavior of strong acids, bases, and salts.

Nearly 2 years before, on August 4, 1885, van't Hoff had written Arrhenius, thanking him for his favorable review of the *Études*, commenting on a memoir by Arrhenius concerning electrolytic conduction, and recapitulating some of the arguments in his Stockholm lecture concerning chemical equilibrium. It was not until March 30, 1887, however, that Arrhenius replied, having waited until he could read van't Hoff's paper in the *Transactions* of the Academy. Now he suggests that the anomalous behavior of electrolytes in aqueous solution may be explained by their dissociation into ions;[30, pp. 219f., 239f.] in this letter he makes what is perhaps his most explicit statement on the matter: "What I called in my paper, 'Sur la Conductibilite' [2nd part], active molecules, are thus the same as dissociated molecules. One of the propositions which I then put forward would now be written:—In all probability all electrolytes are completely dissociated [into ions] at the most extreme dilution."[31, p. 1392; 30, p. 241]

With this explanation available to him, van't Hoff writes, in this, his third paper on osmotic pressure, which appeared in the October 21 number of the *Zeitschrift*,[24, 32] "It may, then, have appeared daring to give Avogadro's law for solutions [equal numbers, equal volumes, equal pressures] such a prominent place [in the theory of solution], and I should not have done so had not Arrhenius pointed out to me, by letter, the probability that salts and analogous substances, when in solution break down into ions." Daring it may have been, daring indeed it was, but he had already committed himself to the gaseous theory, electrolytes notwithstanding, in the *Études* of 1884 and the Stockholm lecture of 1885.

* * *

The question may be raised as to which man, van't Hoff or Arrhenius, stood in greater debt to the other. Without the dissociation theory the colligative properties of solutions treated by van't Hoff could not be rationalized because "most" compounds did not conform with the gaseous theory; on the other hand Arrhenius had not dared go all the way on the dissociation theory until this theory had found support in the observations on osmotic pressure, etc., which had been colligated by van't Hoff.*

We may but briefly turn back to the history of Arrhenius' idea. The study of electrolysis and electrolytic conduction had had a long history which cannot be encompassed here beyond mentioning a few outstanding names, such as Grotthus, Faraday, Clausius, Williamson, Hittorf and Kohlraush—the last-named, with his students, having perfected the use of alternating current and the Wheatstone bridge for the measurement of electrical conductivity of solutions. The necessary data were available for reinterpretation by 1870, but the ultimate idea of the complete dissociation of the solute had been effectively blocked by the long-held conviction, basic to the atomic theory itself, that the 2 atoms of NaCl, for example, are held together by powerful electrical forces and can be separated into "charged radicles" only by the application of a substantial electrical current; and also by the properties of pure metals such as

*To say that Arrhenius' theory "was a direct outcome of van't Hoff's osmotic pressure studies,"[78, p. 111] is to ignore the long history of electrolytic conduction and other interpretations which had been advanced before Arrhenius' time.

Van't Hoff had read Arrhenius' 2 papers[33, 34] of 1884, but the vague notion of "active" and "inactive" particles in solution served only to supply him with a new approach to the mass law, electromotive force and related matters. The fact that the partial dissociation of an electrolyte, such as NaCl, into 2 electrically active particles would increase the number of osmotically active particles was Arrhenius' contribution.

odium, and of pure gases such as chlorine, hich were irreconcilable with the properties f a solution of NaCl.

Arrhenius had begun his studies on the elec rical conductivity of solutions at Upsala un ler the organic chemist, Per Theodor Cleve, ut his ideas were never popular at Upsala nd his major work was carried out in Stock holm between 1881 and 1884 under Erik Ed ung, Professor of Physics to the Swedish Academy. Beyond his own observations, nu merous physical-chemical data were available o him by 1883, all of which called for some unifying interpretation, but in his thesis sub mitted at Upsala in 1883[33, 34] he had handled the problem of electrical conductivity cau tiously by referring (in Clausius' terms) to "active" and "inactive" forms of the solute, only the "active" form serving to conduct the electric current. No explanation of "activ ity" is given nor is it stated why "activity" increases with dilution, and the word "disso ciation" is not mentioned. The older inter pretations were reworked, quantified, and given new applications, but without a com plete break with tradition. Even so, the thesis was heterodox enough to bring opprobrium upon its author, who was granted his degree (1884) *non sine laude approbateur,* the double negative indicating in Sweden that he was just "a little better than passing."[31]

Only after reading van't Hoff's paper, and noting the anomalous behavior of electrolytes in respect to osmotic pressure, etc., did Arrhenius break with tradition and state that in infinitely dilute solutions electrolytes are completely dissociated, and that van't Hoff's law for osmotic pressure, and indeed all the colligative laws, apply to all substances, non electrolytes and electrolytes, if partial or com plete dissociation is taken into account.

The idea that some of the molecules of a olute may, when in solution, undergo com lete dissociation for a finite time into ions hich have virtually complete electrical and hemical independence went considerably be ond the undefined term "active." It was (in ffect) this idea which, as Arrhenius later aid, occurred to him "on the night of the 17th of May in the year 1883,* and I could not sleep that night until I had worked through the whole problem."[8, p. 115]

Only after he had read van't Hoff's Stock holm lecture in 1886, however, did he venture to publish 2 papers in Sweden[35, 36] and to write his definitive paper in German,[37, 24] the last appearing shortly after van't Hoff's paper in volume 1 of the new *Zeitschrift.* The *Zeitschrift* elicited a mixed reaction on the part of the scientific public—between van't Hoff and Arrhenius, so it seemed to some, it had gotten off to a bad start. Though the gas eous theory of solution was soon accepted, the theory of electrolytic dissociation was for many years opposed by every seemingly ra tional, and many irrational arguments, except by Ostwald, who championed the new theory from the start. Once when Ostwald visited Upsala, Cleve asked him ". . . and you are also a believer in these little sodium atoms swimming around?"[8, p. 117] It was unthinkable that the bland action of water as a solvent could cause a molecule to separate into its electrically charged atoms—and one imagines that there were those who hesitated to wash their hands for fear that electric sparks might jump up to their fingertips.

* * *

It has been said that, to van't Hoff, his osmotic theory meant chiefly a new and con venient way to determine molecular weights of dissolved substances. I do not believe it. Van't Hoff was less interested in molecular weights than in chemical dynamics, chemical affinity, and a rational theory of solution. In retrospect, I regret that he abandoned the con sideration of experimentally measured vapor pressure for the circuitous reasoning of the Clausius-Carnot cycle because thereby the theory of solution lost many years. Yet van't Hoff and Arrhenius—we will not attempt to judge the relative merits of their contribu tions—between them started us along the road we travel now.

*His thesis was presented to the Academy on June 6 and must have been completed some time before.

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Protoplasm

Protoplasm is a system of exquisite sensitiveness. In order that it may survive it must be protected from too great, or too rapid, or too irregular fluctuations in the physical, physico-chemical, and chemical conditions of the environment. Stability may sometimes be afforded by the natural environment, as in sea water. In other cases an integument may sufficiently temper the external changes. But by far the most interesting protection is afforded, as in man and higher animals, by the circulating liquids of the organism, the blood plasma and lymph, or, as Claude Bernard called them, the milieu intérieur. In his opinion, which I see no reason to dispute, the existence and the constancy of the physico-chemical properties of these fluids is a necessary condition for the evolution of free and independent life. This theory of the constancy of the milieu intérieur was an induction from relatively few facts, but the discoveries of the last fifty years and the introduction of physico-chemical methods into physiology have proved that it is well founded.—L. J. Henderson. *Blood. A Study in General Physiology.* New Haven, Yale University Press, 1928, p. 20.

The Structure of Water and Electrolyte Solutions

By RICHARD J. PODOLSKY, PH.D.

Intermolecular forces tend to organize water molecules into a tetrahedral lattice, conferring "crystalline" properties on the liquid. The simple ions, such as Na^+, K^+, and Cl^-, have dimensions which are of the same order as the water molecule. Therefore, in aqueous solution, ions can substitute for water molecules in the water lattice. However, because the force between ion and water molecule differs from the force between 2 water molecules, the properties of the lattice are perturbed by an ion. Transport processes in electrolyte solutions are analyzed in terms of the *specific* lattice perturbation produced by each ion. This approach is contrasted with the classical hydrodynamic model in which ions in solution are treated as spheres in a continuous fluid. The relevance of ionic transport in aqueous solution to the transport across biologic membranes is briefly discussed.

Theory of Dissociation

ABOUT 70 years ago Arrhenius invented the theory of dissociation to account for the conduction of an electric current through an electrolyte solution. In his day the textbooks taught that electrolytes were neutral molecules which decomposed into charged particles *as a result of* the applied voltage. However, while thinking about the measurements of conductance which he was making for his doctor's thesis, it occurred to Arrhenius that the charged particles that make a solution conduct might exist *before* current passed through the solution. This would mean that an electrolyte dissociated into ions the moment it dissolved, and that these ions would carry an electric current when driven by an applied voltage.

The theory of dissociation worked well in explaining several important chemical problems of the day. For example, van't Hoff was studying the osmotic pressure and the lowering of the freezing point of solutions. He discovered that a nonelectrolyte produced an osmotic pressure, or lowered the freezing point, according to the number of molecules in the solution. But electrolytes were anomalous; they had a much greater effect than expected on the basis of the number of molecules. By taking dissociation into account and calculating the effect according to the number of particles in the solution, the difference between electrolytes and nonelectrolytes disappeared.

The question of why electrolytes dissociated was sidestepped by Arrhenius. A few years later, though, Thomson, and then Nernst, correlated dissociation with the dielectric constant of the solvent. They pointed out that the forces between charged particles would be considerably reduced in a medium of high dielectric constant. Specifically, according to Coulomb's law, the force between 2 charges is inversely proportional to the dielectric constant of the intervening medium. Thus in water, with a dielectric constant of 80, the force between ions will be 80 times less than in the crystal, and there will be a strong tendency to dissociate. This argument made the phenomenon of dissociation seem more sensible, and the Arrhenius theory was generally accepted at the close of the nineteenth century. Biologists were quick to see its implications, and this symposium shows how fruitful a good idea can be.

Hydrodynamic Model for Ionic Transport

Having worked out the general mechanism of electrolytic conductance, it was natural for biologists to try to account for the properties of specific ions. Thinking centered on aqueous solutions since water is the most common solvent. At first a hydrodynamic model was used. In analogy with macroscopic spheres in liquids, an ion was thought of as a charged particle embedded in a continuous fluid. When a macroscopic sphere passes through a liquid, a frictional force develops which is propor-

From the Naval Medical Research Institute, Bethesda, Md.

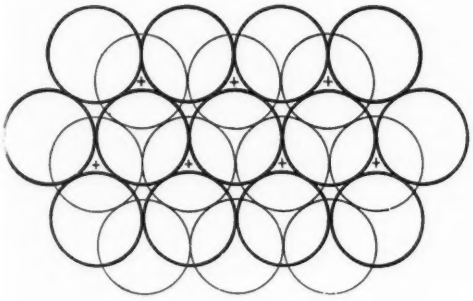

Figure 1

The closest packing of spheres. (Republished by permission of Interscience Publishers.[2a])

tional to its size: the larger the sphere, the greater the drag. It did not seem unreasonable to suppose that the different mobilities of the ions could be explained in terms of the sizes of the particles in solution. However, this approach soon led to difficulties, largely because the size of simple ions is of the same order as the size of the water molecule. From the ionic point of view, water is by no means a continuous fluid.

Studies of fluidity provide the clearest evidence that the ions in a solution cannot be regarded simply as a collection of small spheres in a liquid. According to classical hydrodynamic theory, adding particles to a liquid can only *increase* the viscosity, that is, make it *less* fluid. The change in fluidity should be proportional to the volume fraction of the added particles.[1] Although this effect is used to measure macromolecules, for particles as small as ions it is apparently swamped by another factor. Solutions of cesium iodide, for example, are *more* fluid than pure water, which is embarrassing for the hydrodynamic model.[2]

Structure of Water

We now know that many of the puzzling properties of ions in water are due to the unusual nature of water. Water is a very unusual liquid; indeed, as we shall see, water is more like a crystal than like a fluid.

What distinguishes an "unusual" from a "usual" liquid? One difference is the way

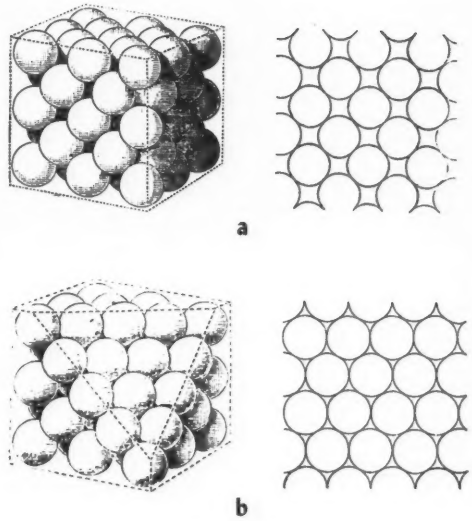

Figure 2

The face-centered cubic lattice. (Republished by permission of Nature.[2b])

the molecules are packed together. In a "usual" liquid the molecules can be treated as hard spheres which interact with each other only on contact. The most stable packing is that which maximizes the number of contacts. The spheres will form layers, 2 of which are diagrammed in figure 1. In the first layer the spheres are in staggered rows. Those in the next layer (thinner lines) fall into valleys shaped by the first layer. Each sphere has 12 nearest neighbors: 6 in its own layer, 3 in the layer above, and 3 in the layer below. This is the *closest packing* for a collection of similar spheres.

This array might be more familiar as the face-centered cubic lattice (figure 2*a*), the arrangement of the chloride ions in the sodium chloride crystal. (The sodium ions are small enough to fit into the spaces between chloride ions.) The layers in figure 1 are revealed by sectioning the lattice at 45°, as shown in figure 2*b*.

The distance between molecules in the liquid state can be measured by x-ray scattering. Knowing this, and the molecular weight, we can easily calculate what the density would

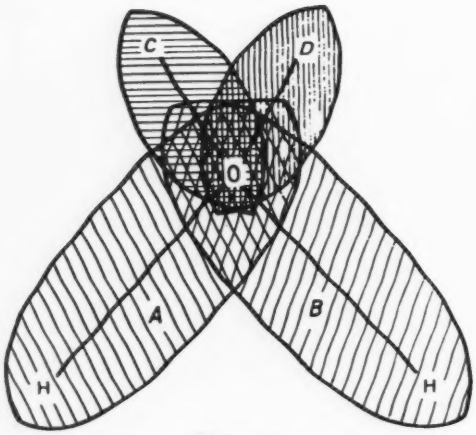

Figure 3

Schematic representation of an isolated water molecule. (Republished by permission of Research.[4])

be if the molecules were closely packed.* Conversely, if the density, calculated on this basis, turns out to be correct, the molecules are indeed closely packed. Such is the case for typical, or "usual," liquids like argon and methane. But for water (2.9 Å between centers) the calculated density is 1.7 Gm./ml., nearly twice the actual density, which means that the molecules are far from closely packed. And, indeed, x-ray data show that the average number of nearest neighbors is not 12, but between 4 and 5.[3]

For the liquid to have this open structure, there must be relatively strong directional forces between neighboring water molecules. These forces arise from the electronic distribution of the isolated water molecule, schematized in figure 3.[4] The 4 pairs of valence-shell electrons in water occupy 4 orbits (the hatched ellipses) which extend out tetrahedrally from the oxygen nucleus, *O*. The 2 pairs of electrons which form the *A* and *B* orbits, the valence-shell electrons, are associated with the OH bonds; the other 2 pairs, *C* and *D*, are, for obvious reasons, called "lone pairs." Largely because the orbits of the "lone pair" electrons

*A cube with an edge of 1 can hold $1/\sqrt{32}\,r^3$ spheres in closest packing, where r is the sphere radius.

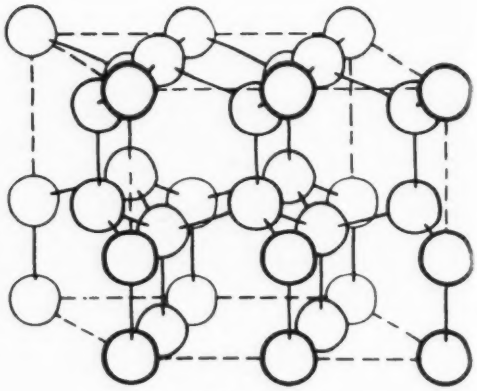

Figure 4

The ideal water lattice. (Republished by permission of Acta Polytechnica [Sweden].[4a])

are directed away from the electrically positive regions of the molecule, the centers of negative and positive charge do not coincide. This electrical asymmetry is the source of the dipole moment of the molecule, which, in turn, is responsible for the electrostatic interaction between water molecules and ions.

Water molecules have a tendency to stick together by forming hydrogen bonds. The bond is due to the interaction of the "lone pair" electrons of one water molecule with the proton of a second water molecule. Since the 4 electronic orbits are tetrahedral, and since each can be involved in a hydrogen bond, it is clear that a population of water molecules will tend to form a tetrahedral lattice (fig. 4). This is the arrangement of the molecules in ice, and, as we know from the low density, much of the structure remains in the liquid state also.

We see, then, that the structured nature of liquid water—called "ice-like-ness" by some authors[2, 5–7]—can be traced back to the fact that each water molecule tends to form hydrogen bonds with 4 other water molecules. Thermal motions lead to rupture of some of the hydrogen bonds, so the degree of structure will depend on the temperature. To the extent that water is ice-like, the molecules can be thought of as distributed among the sites of a tetrahedral lattice.

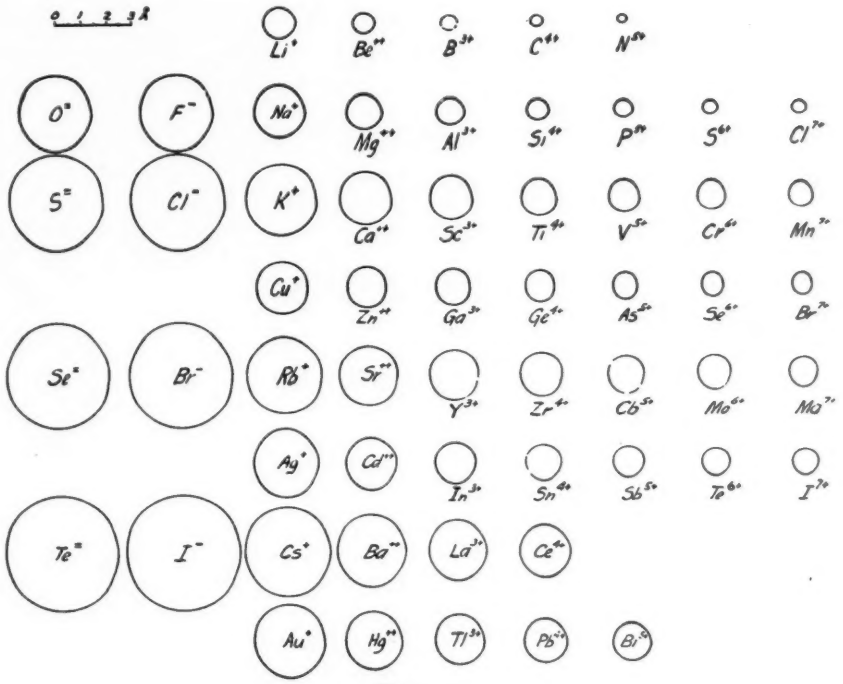

Figure 5

The crystal radii of the atomic ions. (Republished by permission of Cornell University Press.[9])

The obvious difference between water and ice—the fluidity—reflects the relative ease with which molecules can jump between lattice sites in the 2 states. In ice, the molecular coordination is so rigid, and the thermal vibrations are so feeble, that jumps between lattice sites are extremely rare. At liquid temperatures, however, enough of the coordination is broken so that the more vigorous thermal motions lead to molecular jumps, or Brownian motion, between lattice sites. In a sense, each molecule is locked in a "cage" formed by interaction with its neighbors. The energy required for the jump, the *activation energy*, is really a measure of the tightness of the "cage" which binds the molecule.[8] The activation energy can be influenced by physical factors, such as temperature and pressure, and, as we shall discuss subsequently, by introducing ions into the lattice.

The lattice structure accounts for many of the curious properties of water. For example, the anomalous increase in density between 0 and 4 C. is due to the better packing of the molecules after some of the structure is melted. Beyond 4 C. this effect is masked by the usual expansion resulting from the greater amplitude of molecular vibration.

Sizes of Ions

Before considering electrolyte solutions, I should like to say a few words about the ions themselves. By assigning a "radius" to each ion, it is possible to predict a great many properties of ionic crystals. For example, the interionic distances in crystals and the crystal energy can be reckoned very accurately by treating the ions as hard balls of the appropriate radius.[9]

For atomic ions the *ionic radius* can be correlated with the position of the element in the periodic table, as shown in figure 5. The radius

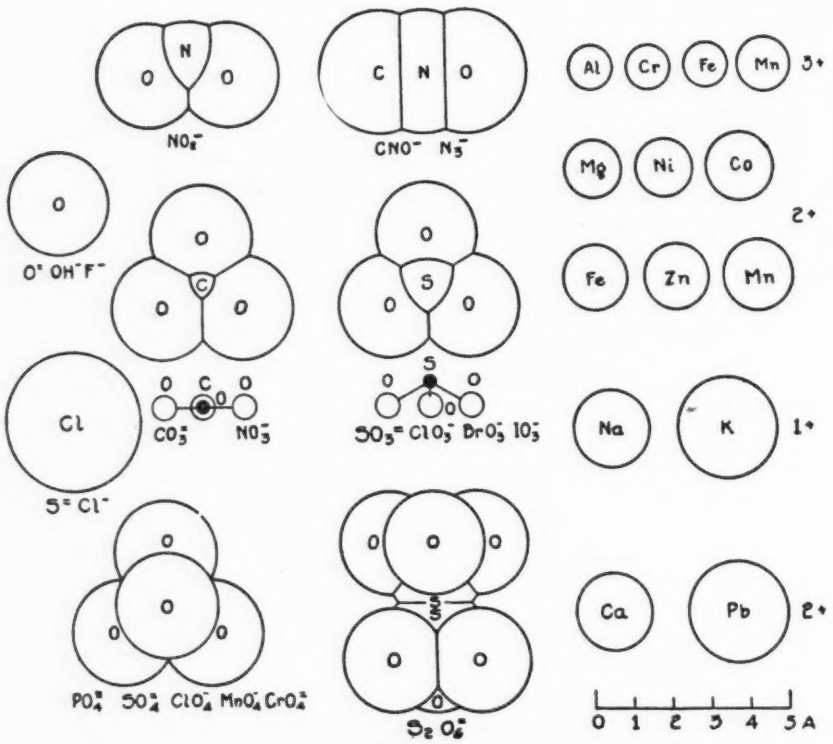

Figure 6

Crystal dimensions of some simple ions. (Republished by permission of Cornell University Press.[9a])

of the circle is proportional to the (univalent) crystal radius of the ion. In a given row, the ions decrease in size, from left to right, because the increasing nuclear charge for the same number of orbital electrons tends to pull the electrons closer to the nucleus. Down the columns, there is an increase in both the number of orbital electrons and the nuclear charge, but since there is no change in the net charge, the radii are somewhat more uniform than across a row.

A water molecule is very nearly the same size as the oxygen ion. Since the fundamental assumption of the hydrodynamic model is that the discontinuities in the solvent are small compared with the size of the suspended particle, and since atomic ions are either smaller than or very nearly the same size as the water molecule, it is not at all surprising that the hydrodynamic model is less than adequate. Even the simpler molecular ions, shown in figure 6 together with some of the biologically important atomic ions, would encounter considerable granularity in moving through water. The forest is lost for the trees.

Lattice Model for Aqueous Electrolyte Solutions

Suppose an electrolyte is dissolved in water. To a first approximation, the ions will occupy the same lattice sites as the water, that is, they will fit into the lattice substitutionally (fig. 7). On the left, a water molecule is tetrahedrally coordinated with 4 neighbors. The black patches locate the hydrogen nuclei. Since the structure is held together by hydrogen bonds, the black patches are opposite the "lone pair" orbits. On the right, an ion is substituted for the central water molecule. This substitution will lead to changes in the water lattice. Since

Figure 7

Water structure and a substitutional ionic solution.

the ion is a monopole rather than a dipole, its electrostatic field will interact with the dipole moment of the water molecules and tend to rotate 2 of the 4 adjacent water molecules. An anion, for example, will attract the positive ends of the adjacent water dipoles toward the center of the tetrahedron, and this rotation will disturb the coordination of the next shell of water molecules. Of course, the influence of the ion on the lattice structure will fall off with distance, and, far from the ion, the lattice will be unperturbed.

Before leaving this figure, I should like to point out that the electrostatic interaction of an ion with water dipoles (in the first shell and beyond) can, in a sense, be compared with its interaction with counter ions in a solid crystal. Carrying this thought further, an aqueous electrolyte solution can be taken as liquid crystal of variable composition. Then dissociation in water would simply be the mixing of 2 compatible crystals, a point of view that might have dissolved some conceptual difficulties of Arrhenius' day.

Transport Processes

It is convenient to think of an ion as a modified water molecule, considerably different in charge, and different in size. These differences will change the activation energy necessary for lattice jumps among sites in the neighborhood of the ion. The magnitude of the change, which depends on the ionic species, will be reflected in a corresponding change in various transport processes. For example, consider an ion that interacts with the adjacent water molecules so that they move about the lattice more easily. Then the self-diffusion of water in the presence of the ion will be greater than in pure water. Also, since the ion itself is locked in a "cage" made up of the water molecules around it, the ion will also move more freely in this case. Conversely, if the ion-solvent interaction is such that water molecules near the ion move with greater difficulty, the ion within the "cage" will also move less freely.

According to this picture, then, both ions and water molecules are distributed among the sites defined by the water lattice. But each ionic species modifies the structure of the lattice around it in a specific way. The activation energy for lattice jumps of both the ion and the water molecules around it, the perturbed water, will be different from that of the unperturbed water, the difference depending on the ionic species. The change in activation energy will be reflected in both the ionic and the water mobility.

The simplest way of measuring the influence of an ion on the water lattice is to measure the fluidity of the solution. All the particles occupying the lattice sites will contribute to the fluidity: the ions themselves, the water

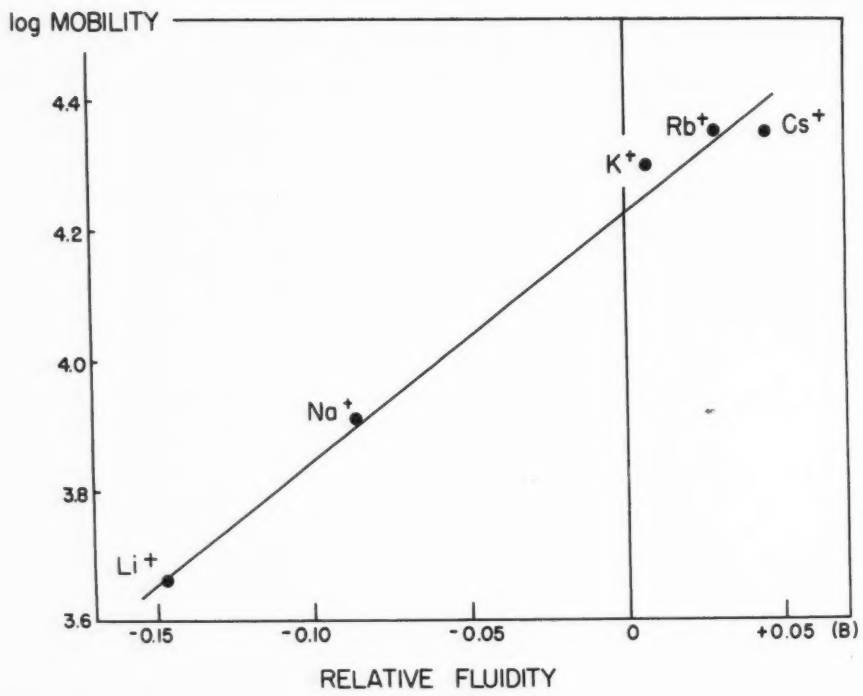

Figure 8

Dependence of the limiting mobility of the alkali ions on the relative fluidity, the Jones and Dole parameter B.

perturbed by the ions, and the water uninfluenced by the ions. If an ion breaks up the structure of the waters around it, the solution will be more fluid than pure water. Conversely, if an ion interacts with the neighboring particles in such a way as to make it more difficult for them to move around, the solution will be less fluid.

This argument can be turned around to use the fluidity of the solution as a basis for calculating the activation energy of *both* an ion and the water molecules with which it interacts.[10] This activation energy can then be used to calculate the mobility of the ions (the electrical conductivity of the solution) and the mobility of water (the rate of diffusion of labeled water through the solution).

Analysis reveals that the ionic mobility should be exponentially related to the fluidity of the solution.[10] To test the result of this analysis, data for the alkali ions have been plotted in figure 8. The abscissa is a measure* of the influence of the ion on the fluidity of water: with an anion that has no effect on the water structure, solutions with cations on the right of zero, e.g., potassium, rubidium, and especially cesium, are more fluid than pure water; sodium, and to a greater extent, lithium, decrease the fluidity of the solution. The ordinate is the logarithm of the corresponding mobility. The data fit the expected linear relation quite satisfactorily. It should also be noted that the smaller crystal radius is correlated with the lower mobility, a point which will be discussed subsequently.

This relation is not restricted to the alkali ions. Figure 9 displays data for 38 different species for which both fluidity and mobility data are available. All the ions have crystal radii less than 4 Å. Three points, in paren-

*The datum used is the Jones and Dole parameter *B*.[10]

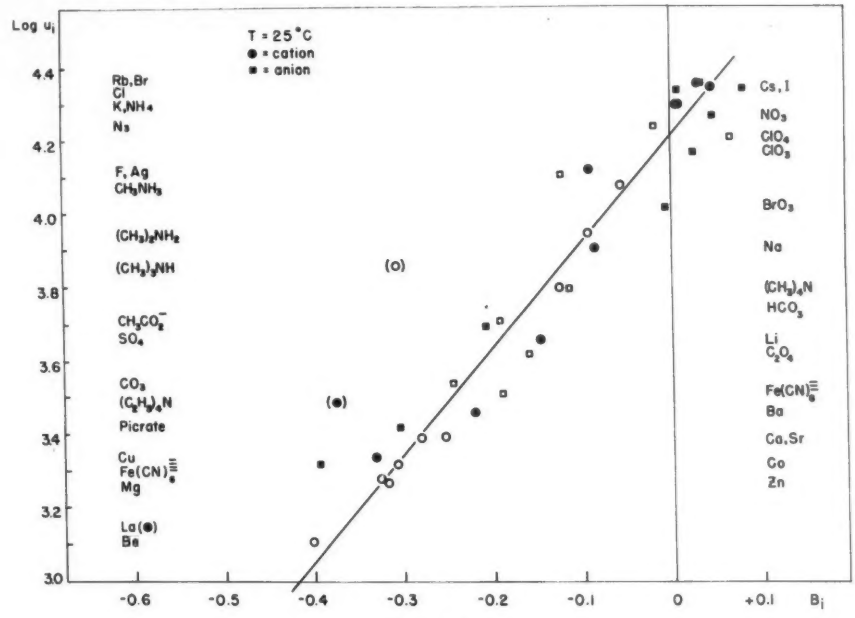

Figure 9

Dependence of the limiting ionic mobility on the relative fluidity, the Jones and Dole parameter B. *(Republished by permission of the Journal of the American Chemical Society.*[10])

theses, trimethylammonium, tetraethylammonium, and lanthanum, are clearly out of line. But the scatter about the expected linear relation does not seem unreasonable, especially since the fluidity data are from many sources, some quite old.

The structure-breaking—or structure-forming—effects of the ions are also reflected in their influence on the self-diffusion of water. In these experiments, the diffusion of labeled water across an electrolyte solution is compared with the diffusion through pure water. Whether the tracer moves through the solution more or less rapidly than in pure water should depend on whether the ions break, or strengthen, the lattice around them, which, as has been discussed previously, can be decided from the fluidity of the solution (fig. 10). The ordinate is the change in the self-diffusion of labeled water, and the abscissa, as in figures 8 and 9, is a measure of the fluidity of the solution. For example, a molar solution of potassium iodide, at 10 C., is about 12 per cent more fluid than pure water at that temperature; labeled water diffuses through it about 13 per cent faster than in pure water. The straight line was calculated from the model;[10] the points are the experimental findings for the 3 salts, KI, KCl, and NaCl, at the indicated temperatures.[11] That the model also works in this case is especially significant since the slope and intercept of the straight line were derived without benefit of adjustable parameters.

Ionic Hydration

Until now, I have purposely avoided the problem of ionic hydration, that is, the question of whether an ion migrates alone or with a number of attached water molecules. The concept of hydration was invented to explain why the ions with smaller crystal radii are generally less mobile than those with larger crystal radii. It was argued that the water dipole would be attracted and locked to the

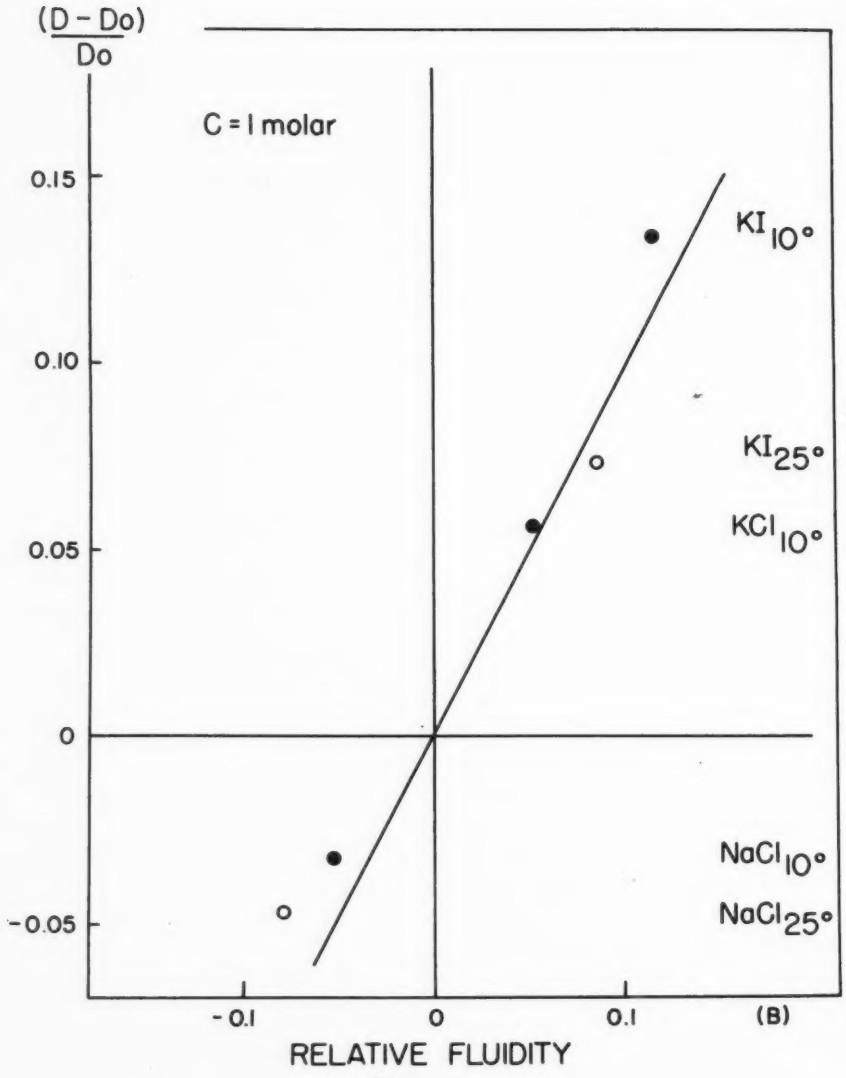

Figure 10

Dependence of D, *the self-diffusion coefficient of water in electrolyte solutions, on* B, *the Jones and Dole parameter. (Republished by permission of the Journal of the American Chemical Society.*[10]*)*

smaller ions since the electric field at the surface of an ion increases with decreasing radius. Then the hydrodynamic unit—the cluster of particles that stick together in Brownian motion—would actually be larger for the ions having the smaller crystal radii. This has always been a murky point in the theory of electrolytes. Direct studies, with tracers, show that the water around small highly charged ions—e.g., aluminum, with a charge of 3, and thallium, with a charge of 4—exchanges, within a few minutes,[12] with mole

cules far from the ion. Whether water sticks to these ions for the shorter times involved in diffusion is not known. If the hydrodynamic model for ionic transport is put aside, the evidence for a rigidly bound shell of water on the smaller ions becomes quite tenuous.

An energetic rather than a geometric factor probably lies behind the lower mobilities of the smaller ions. The interaction of the ionic field with the dipole moment of the adjacent water raises the activation energy which is necessary for motion of these waters. This effect can be large, compared with structure breaking from disruption of the water lattice, in the high fields of the surface of the smaller ions. If the *net* result were a tighter "cage" around the ion, the ion would have a correspondingly lower mobility.

Conclusion

I think the lesson to be drawn from the success of the lattice model in understanding transport processes in electrolyte solutions is that the analysis starts with the *change* in the solvent due to the ion. This is a turnabout from the earlier hydrodynamic model in which the solvent properties were supposed to be uninfluenced by the ion and the differences among ionic solutions were attributed to the "sizes" of the ions in solution, an elusive property which could never be pinned down. In the lattice model, on the other hand, the water structure is *changed* in a specific way by each ionic species. This change depends on the way the lattice adjusts to the differences between the force field of an ion and that of a water molecule, or, more simply, on how the ion "fits" into the water lattice.

With regard to membrane transport, the relative mobility of ions in the membrane and in water will be the same to the extent that ions move through "wide" channels containing water with lattice properties like pure water. However, if the water lattice is modified by the membrane, as would happen in the case of "narrow" pores, there is no direct way of relating transport in the membrane to transport in water. As in the case of water, the dominant factor is probably the reciprocal interaction of ion and solvent. This interaction might be the reason for some of the difficulties encountered in explaining membrane specificity in terms of either crystal radius or ionic mobility in water.

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Protein Molecules in Solution

By IRVING M. KLOTZ, PH.D.

A protein molecule in solution may undergo a host of interactions: with solvent, with small molecules and ions, with itself, with other macromolecules. In a physiologic milieu, all of these possibilities are present. A variety of interactions will occur. On the basis of the behavior of these substances in simple systems, reasonable guesses can often be made as to which interactions will predominate in a biologic environment.

IN THIS paper, we shall examine some of the properties, and the behavior, of proteins in solution. Our view will be a molecular one and we shall interpret the behavior of proteins in terms of the structure of these macromolecules and their interactions with their environment. It is appropriate, therefore, to summarize briefly at the outset, some of the salient features of the structure of protein molecules.

Brief Review of Protein Structure

Proteins are supermolecules made up of small amino acid building blocks. The chains of amino acids are held together by peptide linkages, each residue contributing about 3.5 Å to the length of the chain (fig. 1). As has long been recognized, specificity of proteins must reside ultimately in the particular sequence of amino acids in the chains of the supermolecule. As a result of the magnificent skills of several individuals, sequences (sometimes called the *primary structure*) are now known completely for 2 proteins, insulin[1] and ribonuclease,[2, 3] and almost completely for several others. The arrangement in insulin is shown in figure 2.

Overwhelming evidence exists that within a protein molecule the polypeptide chain does not occur as a stretched-out string of residues. While the basic configurations assumed by the chains are not yet established unequivocally, and certainly must differ among different classes of protein, the best model so far is the α-helix coil suggested by Pauling, Corey, and Branson[4] (fig. 3). With such a coiling, it takes approximately 7 amino acids to traverse 10 Å

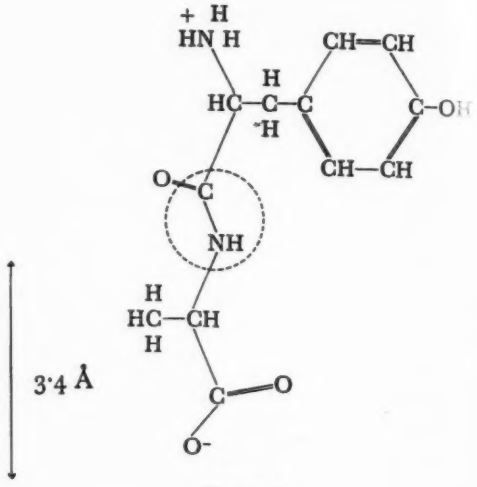


Figure 1

Peptide bond (in broken-line circle) between 2 amino acids, tyrosine (top) and alanine (bottom). Scale in angstrom units is shown at left.

in the direction of the axis of the helix. In a helix, furthermore, a given amino acid has as neighbors not only the 2 amino acids to which it is attached by peptide linkages, but also those residues 1 turn in the helix above or below. If these steric relationships were known, and as yet they are completely obscure, we would say that the *secondary structure* of the protein molecule had been established.

If the basic configuration of the polypeptide is a helical coil, then it is clear from the known dimensions of globular proteins that these helices must be folded over in various configurations so as to be contained, in most cases, in a volume which is very roughly spherical in shape. Such conformations have usually

From the Department of Chemistry, Northwestern University, Evanston, Ill.

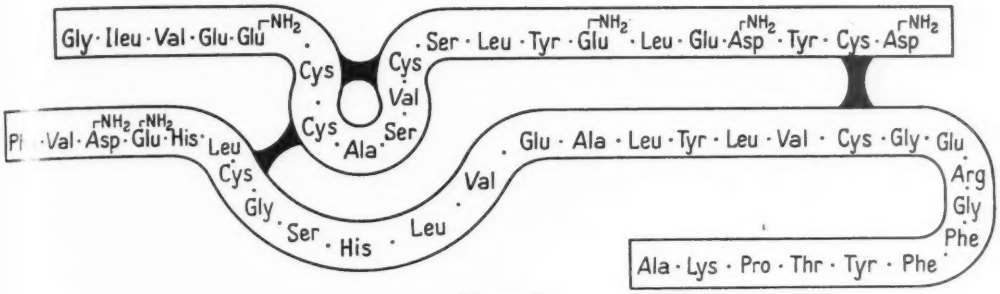

Gly · Ileu · Val · Glu · Glu
Cys
Cys
Val
Cys
Ala
Ser
· Ser · Leu · Tyr · Glu · Leu · Glu · Asp · Tyr · Cys · Asp
Val · Asp · Glu · His · Leu
Cys
Gly
Ser
His
Leu
Val
Glu · Ala · Leu · Tyr · Leu · Val · Cys · Gly · Glu
Arg
Gly
Phe
Ala · Lys · Pro · Thr · Tyr · Phe

Figure 2

Sequence of amino acids in insulin. There are 2 peptide chains linked together by disulfide bonds represented by heavily blackened parts in the diagram. Gly = *glycine;* Phe = *phenylalanine; etc. (Republished by permission of Naturwissenschaften.*[37])

been called the *tertiary structure* of the protein molecule. Despite heroic efforts over an extended period, information concerning 3-dimensional configuration is available for only 1 protein, myoglobin,[5] (fig. 4) which shows a very complicated pattern of foldings, the biologic significance of which is not comprehended at all.

Nevertheless, even in the absence of detailed structural models, we do have extensive knowledge of the over-all sizes and gross shapes of many protein macromolecules. A few of these are shown in figure 5. It is only from these very gross models that we can begin our considerations of the properties of protein molecules.

Interactions of Protein Molecules with Solvent

In any discussion of the behavior of proteins in solution, i.e., of the interactions of these macromolecules with their environment, we recognize that the surfaces shown in figure 5 cannot actually be as smooth as the drawings might imply. If the figure were expanded sufficiently we would be able to visualize the specific side-chains of the individual amino acid residues. A few polar side-chains are shown schematically in figure 6. Such polar and nonpolar substituents play a vital role in the interaction of the macromolecule with its environment.

That the environment of the protein surface is very different from that in the bulk of the solution is evident from the marked differences in the chemical behavior of functional

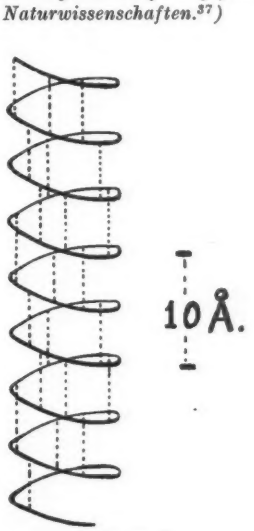


Figure 3

Schematic diagram of helical configuration of a polypeptide chain (solid line) with hydrogen bonds represented by broken lines.

groups which are attached to protein side-chains. For example, sulfhydryl groups in simple compounds, such as glutathione, react rapidly and completely with many substances, particularly heavy metals:

$$R - SH + Ag^{+} = R - SAg + H^{+}. \quad (1)$$

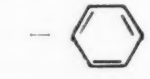
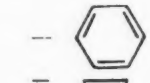
The same −SH group attached to a protein (for example, β-lactoglobulin) acts as if it were completely hidden from any attacking reagent, such as Ag^{+} (fig. 7). The diffusion current of Ag^{+} added to protein in buffer rises just as it would if protein were absent. Only if the protein is denatured, for example with

Figure 4

Configuration of myoglobin. The gray disk is the heme group, the little black ball attached to it, an artificially introduced group required for the x-ray analysis of this macromolecule. The white parts are the polypeptide configuration. (Republished by permission of Endeavour.[38])

Table 1

Some Unusual pK's in Proteins

Protein	Group	pK Expected	pK Found
Ribonuclease		10	9.9 (3 groups) >11.5 (3 groups)
Ovalbumin		10	>12
Hemoglobin		6.5	< 5

ura, does the −SH group become visible to the Ag^+. Denaturation is said to "unmask" the mercaptan in the protein molecule.

Similarly, if we examine the titration characteristics of acid-base groups of a protein, we often find peculiarities (table 1). For example, the phenolic −OH groups of tyrosine residues often will not dissociate H^+ ions at pH values much higher than are characteristic for this substituent.[6, 7] Also, basic residues, such as histidine, may not accept a hydrogen ion until the pH is lowered much below that at which an imidazolium ion is normally formed.[8] A more detailed description of such unusual acid-base behavior is shown in figure 8 for an $-N(CH_3)_2$ group which has been artificially introduced into a protein; hydrogen ions are taken up only at much stronger acidities than are necessary when the same group is attached to a simple amino acid.[9]

There is thus no question that a protein environment may modify the properties of a substituent. However, the molecular basis of the changed behavior is less certain. I should like to present primarily our views[10] on this subject.

As we have suggested recently, many aspects of the behavior of proteins in solution can be interpreted advantageously if we assign some special properties to the water in the immediate neighborhood of the macromolecule. There are good reasons, aside from the properties of proteins as such, for assuming a special ice-like character of regions of the hydrate water of these macromolecules. As was mentioned previously, proteins contain a large number and variety of nonpolar side-chains (table 2); in simple molecules these side-chains possess the remarkable property of forming crystalline hydrates consisting predominantly of molecules of water in an ice-like lattice with occasional holes into which a nonpolar molecule may fit.[11, 12] Such nonpolar hydrates are stable at temperatures well above the melting point of ordinary pure ice; it is the presence of the nonpolar molecules in the holes of the polyhedrons (figs. 9 and 10) formed by the H_2O molecules which stabilizes the ice-like lattice.

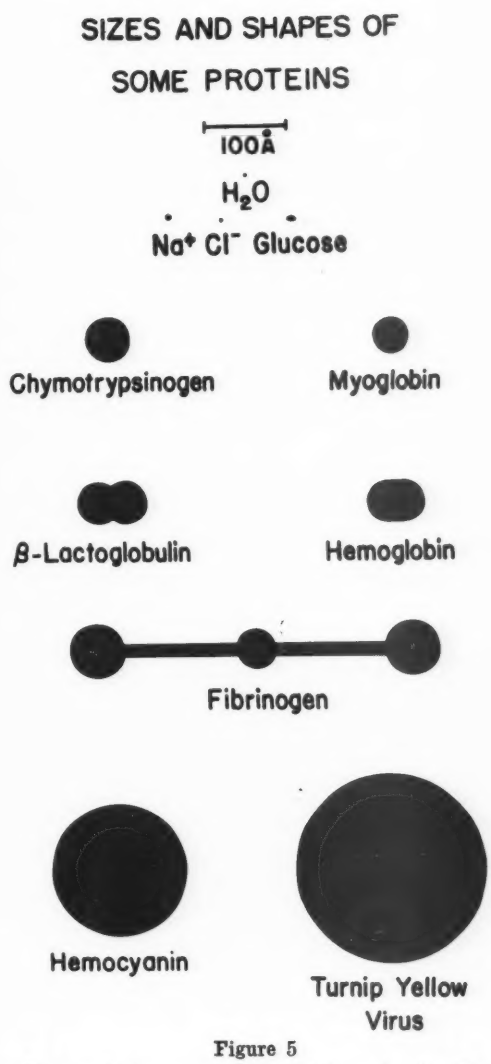


Figure 5

Sizes and shapes of some protein molecules. For comparison, some small molecules and a scale are shown at the top.

In a protein molecule many nonpolar side-chains exist in juxtaposition. Thus, there could be a "coupling" of the ice-like lattices which are formed around each residue with a consequent strengthening of the resultant hydrate structure. The structure of the lattice which is formed by the coupling of water of hydration of protein side-chains may not be

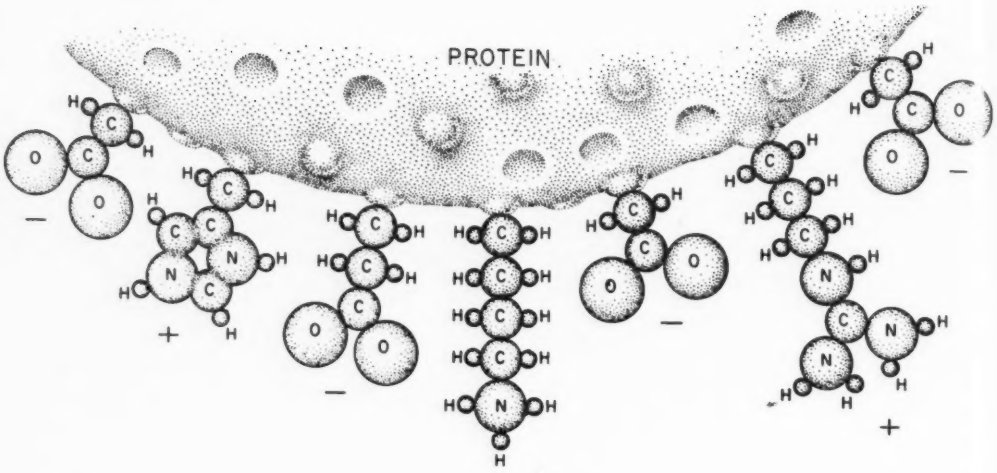


Figure 6

Schematic diagram of some ionic side-chains in protein molecules.

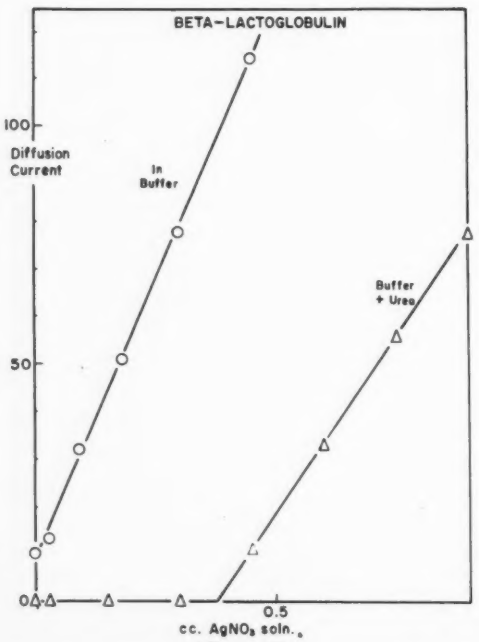


Figure 7

Detection of –SH groups by amperometric Ag^+ titration of protein β-lactoglobulin. In buffer, current due to free Ag^+ goes up immediately upon addition of $AgNO_3$, i.e., *–SH groups on protein are not detectable. In buffer + urea, current stays at zero for substantial addition of Ag^+, a behavior due to removal of Ag^+ by –SH of protein.*

Table 2

Comparison of Amino Acid Side-Chains with Molecules Which Form Hydrates

Some amino acid side-chains		Some molecules which form crystalline hydrates
$—CH_3$	(Ala)	CH_4
$—CH(CH_3)_2$	(Val)	$CH_2(CH_3)_2$
$—CH_2—CH(CH_3)_2$	(Leu)	$CH_3—CH(CH_3)_2$
$—CH_2—SH$	(Cys)	$CH_3—SH$
$—CH_2—CH_2—S—CH_3$	(Met)	$CH_3—S—CH_3$
$—CH_2—C_6H_5$	(Phe)	C_6H_6

the same as that of nonpolar hydrates of small molecules, such as those listed in table 2, but the possibilities for cooperative interaction in the macromolecule should be even greater, since the residues are even initially relatively fixed in position by the framework of the protein molecule. A very schematic and exaggerated representation of such an ice-like lattice is shown in figure 11.

The presence of patches or ribbons of ice-like coverings around various regions of a protein molecule would affect both the kinetics of

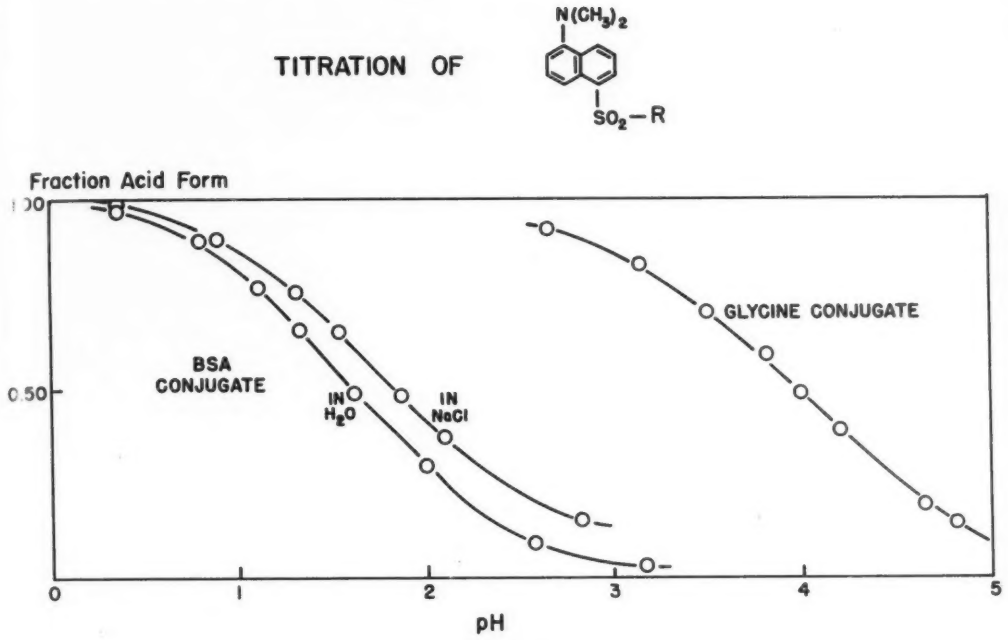


Figure 8

Uptake of H^+ as a function of pH by dimethylamino group (of molecule shown) when attached to glycine and bovine serum albumin (BSA), *respectively. A much lower pH, i.e., stronger acidity, is required to place H^+ on conjugate to the protein.*

its reactions and its state of equilibrium. Rates of diffusion of essentially all substances to a reactive site under an ice layer would be greatly reduced. With Li^+, for example, it is known[13] that the rate of diffusion in ice is at least 10,000 times slower than in liquid water. On this basis, one can readily understand why Ag^+ titrates very slowly with —SH residues in many proteins, e.g., β-lactoglobulin (fig. 7). Judging from the observation that mercaptans form crystalline hydrates, we might conclude reasonably that —SH groups in proteins could participate cooperatively with neighboring side-chains in the formation of an ice patch. Diffusion of Ag^+ through this ice patch would be greatly hindered as compared to the movement of this ion in ordinary water. Consequently, the protein mercaptan acts as if it were masked.

Similarly, the shift in the titration curve of protein-bound groups such as $-N(CH_3)_2$ toward a lower pH (fig. 8) can be readily interpreted. Such a shift means, in essence, that the environment is more favorable to $(CH_3)_2N$ than to $(CH_3)_2NH^+$. Such a bias seems very reasonable, for the creation of a charged group in place of the uncharged one would require some breakdown of the ice lattice of the protein.

Interactions of Protein Molecules with Small Molecules and Ions

In addition to interacting with solvent, proteins may also interact with all types of small ions and molecules in the solution. The stability of the complexes which are formed depends largely upon the character of the macromolecule and the small ligand, but it is also affected by the nature of the environment in the solution. An exhaustive survey of these factors would be inappropriate for this symposium, but a few representative examples of some dominant factors in these interactions will be described.

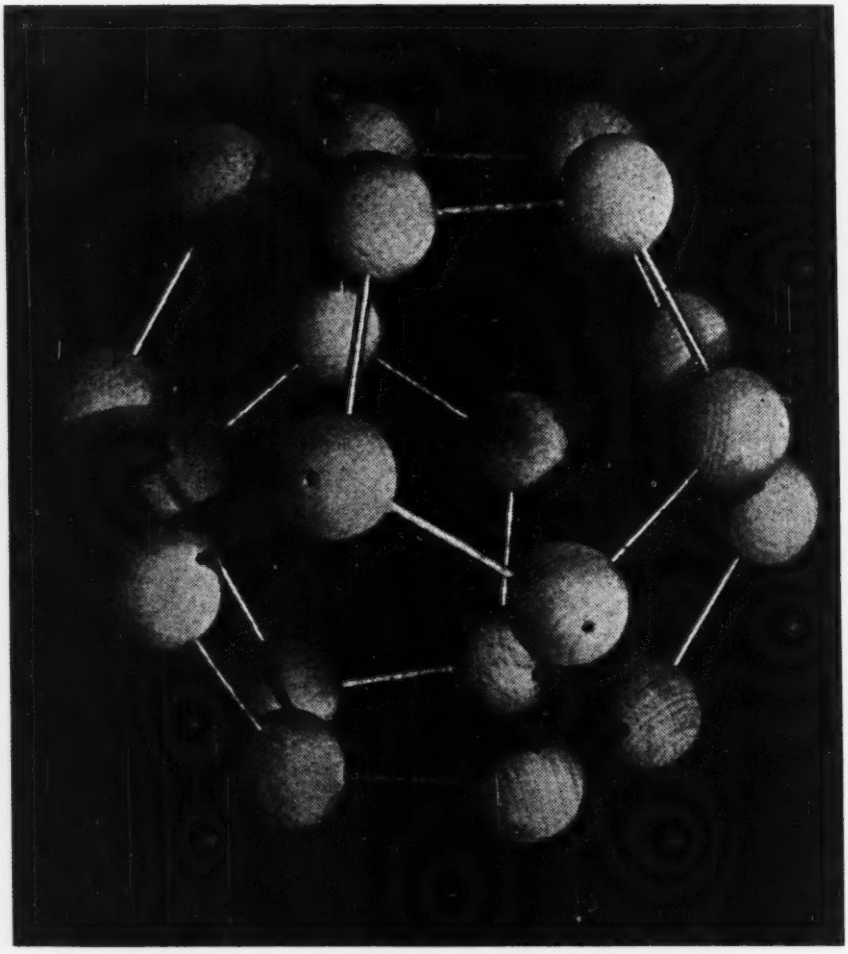


Figure 9

Model of arrangement of water molecules in crystalline hydrates of nonpolar molecules. Each ball represents 1 water molecule. The molecules are arranged in pentagonal planes, 12 of which enclose a dodecahedral space. The hole inside this polyhedron is about 5 Å in diameter. (Republished by permission of Zeitschrift für Elektrochemie.[11])

Metal Ions and Hydrogen Ions

Figure 6 was introduced into this paper largely to show some polar side-chains which occur in practically all proteins and which are capable of forming complexes with many metal ions. Practically all metal ions form complexes with some protein side-chains. Nevertheless, there are differences in specificity among the metals; some examples of these differences are summarized in table 3. In general, the alkali metals (e.g., Na^+) do not form complexes, although with special proteins (e.g., myosin[14]) binding is observed, probably due to a clustering of appropriate residues of the macromolecule. The alkaline-earth metals (e.g., Ca^{++}) form complexes largely with carboxylate or phosphate side chains. Heavier metals, particularly of the transition series, are bound to practically all groups of the protein which are capable of

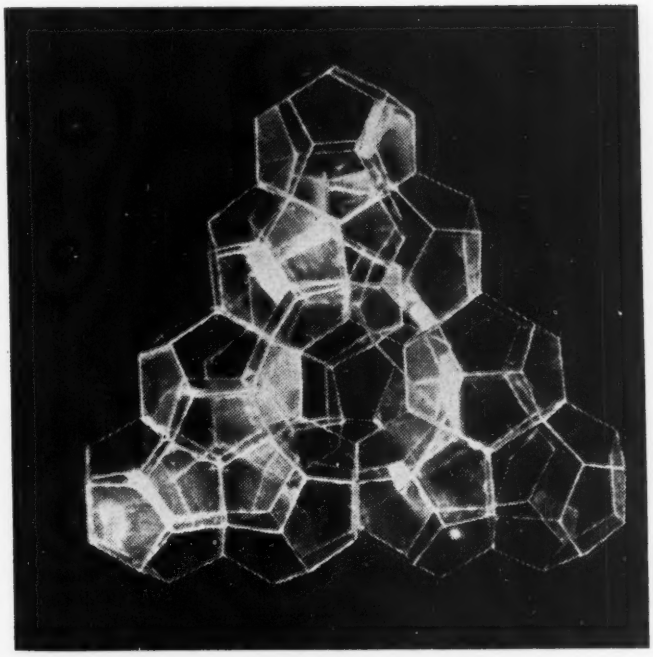


Figure 10

Polyhedrons of type of figure 9 arranged to form a large lattice, as is found in crystalline nonpolar hydrates. Compartments of 5, 6 and 7 Å are available for enclosure of nonpolar molecules within water structure. (Republished by permission of Oxford University Press.[39])

Table 3

*Binding of Metal Ions by Some Substituents in Proteins**

	HO—P(=O)(—O—)—	—C(=O)—O—	—HC=C—HN—C(—N)—H	—HNH—	—O—(C6H5)	—S—
Na^+	0	0	0	0	0	0
Ca^{++}	+	+	0	0	+	0
Fe^{+++}	+	+	+	+	++	+
Zn^{++}	+	+	+	+	+	+
Cu^{++}	+	+	+	+	+	++
Hg^{++}	+	+	+	+	++	++

*For further quantitative estimates of affinities, see Gurd and Wilcox.[13a]

interacting with H^+. Nevertheless, there are differences in specificity. Beyond the very qualitative information of table 3, one can point to the very high affinity of Fe—much greater than that of Cu—for phenolic oxygens

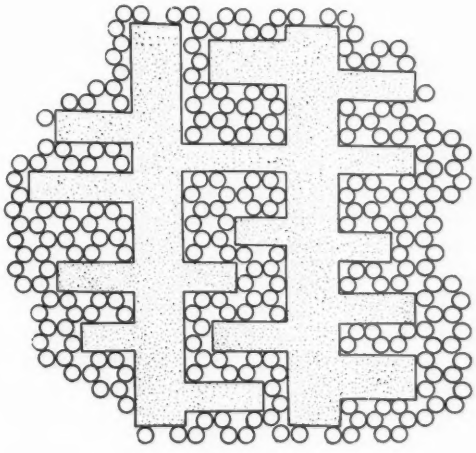


Figure 11

Schematic diagram of ice-like character of hydration sheath around nonpolar groups in protein molecules. Small circles represent water molecules.

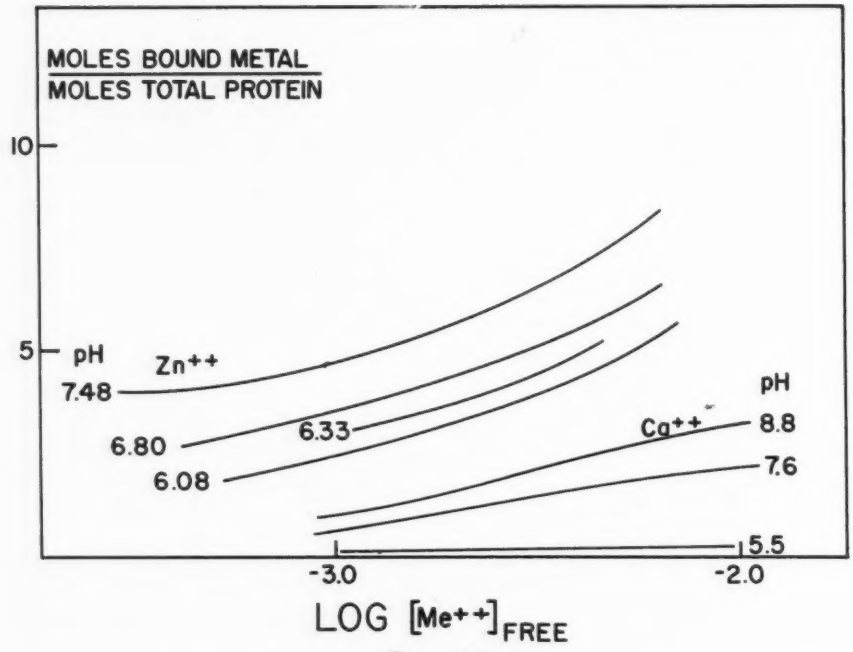


Figure 12

Uptake of Zn^{++}, and of Ca^{++}, respectively, by serum albumin at different pH's. Ordinate represents number of Zn^{++} (or Ca^{++}) ions bound by 1 molecule of protein; abscissa gives the logarithm of the concentration of free metal ion (in water) in equilibrium with the bound metal ion on the protein.

in compounds such as tyrosine. On the other hand, Cu is bound more strongly to amine nitrogens or to mercaptan groups. An interesting case not shown in table 3 is Pb ion which, like Zn or Cu, is bound strongly to mercaptan groups. On the other hand, Pb shows a much greater affinity for carboxyl than for amine groups; the opposite preference is characteristic of Zn and Cu.

There are also specificities among protein molecules, although these are not too well understood in molecular terms, except perhaps for the heme pigments in which a definite prosthetic group can be isolated. Thus, conalbumin or transferrin shows a remarkable affinity for Fe, the interaction occurring with tyrosine residues;[15] yet, many other proteins have a substantial complement of tyrosines, but no unusual avidity for Fe.

The ligand groups shown in table 3 are all capable of binding H^+ ions. It is not surprising, therefore, that H^+ ions should compete with metal ions and, hence, that the binding of metals is dependent on the pH. For example, Fe(III)-conalbumin is stable above pH 7 but loses Fe(III) below pH 7 (if citrate is present[15]). More detailed results for albumin complexes with Zn[16] and with Ca[17] are illustrated in figure 12. In all cases, lower pH, i.e., increasing acidity, lowers the amount of bound metal ion.

The extent of binding of metal ions by proteins may also be controlled in part by "metal buffers," i.e., complexing and chelating agents with a strong affinity for the cation. These agents thus compete with the protein for the metal. For example, the binding of zinc ions by serum albumin at pH 6.4 can be reduced by addition of glycine,[18] which forms complexes with the metal. Similarly, acetate competes with protein for lead ions, iodide for mercury,[18] citrate for copper.[19] These "metal

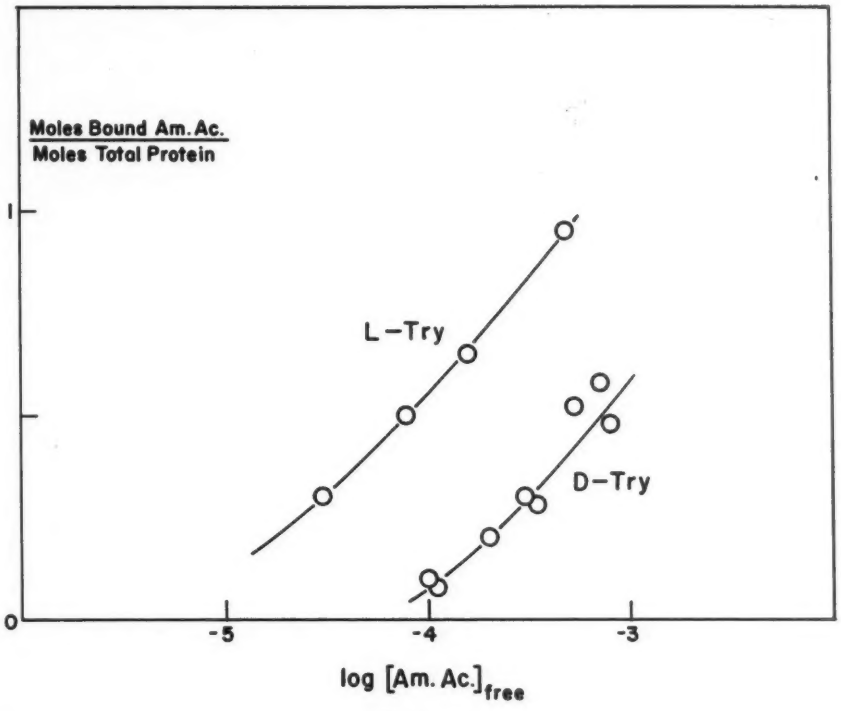


Figure 13

Comparison of the extent of binding of L-tryptophan with that of D-tryptophan by serum albumin. Ordinate gives number of bound amino acid molecules on each protein molecule, and abscissa shows concentration of free tryptophan in equilibrium with that bound to protein.

buffers'' can be used to control the extent of binding of cations and, hence, the physical or biologic consequences of such binding.

Organic Molecules

Proteins also form complexes with organic ions and molecules. For purposes of illustration, we shall consider just a few complexes of serum albumin. In a sense, this protein is not representative because it binds a great variety of molecules and ions whereas one usually emphasizes the high specificity of the interactions of proteins, e.g., enzymes or antibodies. We shall not discuss the molecular interpretations of such specificity. It should be pointed out, however, that serum albumin also shows remarkable discriminatory properties. A most striking illustration occurs with tryptophan (fig. 13), the L-isomer being bound substantially, the D-isomer markedly less so.[20] Preferences also are shown for positional isomers. For example, albumin distinguishes between o-, m-, and p-methyl red (fig. 14), not only with respect to affinity but also, as judged from spectroscopic manifestations,[21] with respect to the nature of the interaction.

As with metal ions, the binding of organic ions by proteins may be decreased by competition of other anions. Thus, the addition of chloride, acetate or phthalate ions, reduces the binding of methyl orange by serum albumin (fig. 15). Obviously, the type of buffer solution used may influence substantially the interaction between macromolecule and small ion. Nonionic organic molecules may also compete with ionic. Thus, decanol reduces the binding of tryptophan,[20] and alcohols, from

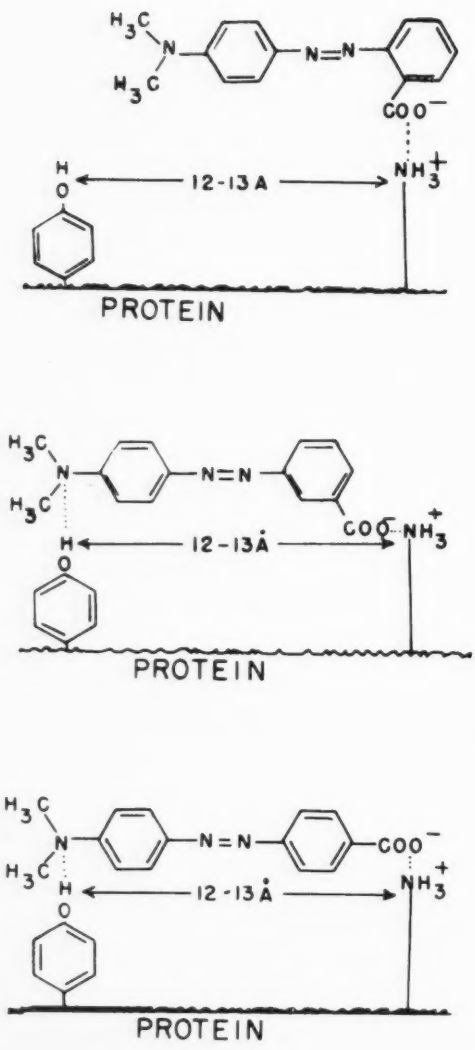


Figure 14

Structural formulas of some positional isomers between which serum albumin can discriminate either in extent of binding or in effect of protein on color of small molecule. Also shown are distances between groups on protein involved in binding of these small molecules, for comparison with distances between $(CH_3)_2N-$ and $-COO^-$ substituents on each small molecule. Protein side-chains are too far apart (12 to 13 Å) to link to both $(CH_3)_2N-$ and $-COO^-$ of top molecule; hence linkage is to $-COO^-$ only, and binding and spectroscopic effects differ in this case from results with lower 2 molecules.

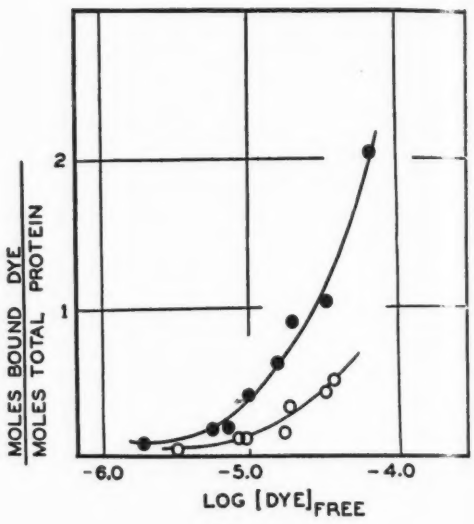


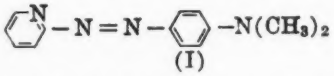
Figure 15

Difference in extent of binding of methyl orange by serum albumin in presence of different buffers: ●, acetate buffer, ○, phthalate buffer. Lower binding curve in presence of latter buffer shows that phthalate ion interferes more than does acetate.

methanol to decanol, reduce the binding of methyl orange by serum albumin.

It is also possible to increase the uptake of organic molecules and ions by a variety of stratagems, 2 of which will be mentioned here.

A molecule such as pyridine-2-azo-p-dimethylaniline (I) is not, by itself, bound appreciably by proteins. However, if very small amounts of metal, e.g., Zn^{++}, are added to the solution, very substantial binding of (I) follows (fig. 16). As we now know, the metal



(I)

ion acts as a bridge between protein and organic molecule (fig. 17). The net result is a strong cooperative interaction. Zinc ion and (I) have a small affinity for each other ($k = 0.2 \times 10^3$) even in the absence of protein; attached to a protein, the affinity of Zn^{++} for (I) rises almost 50-fold ($k = 9 \times 10^3$).

Equally striking increases in the binding of anions by protein molecules are observed in the presence of glycine (fig. 18), but here

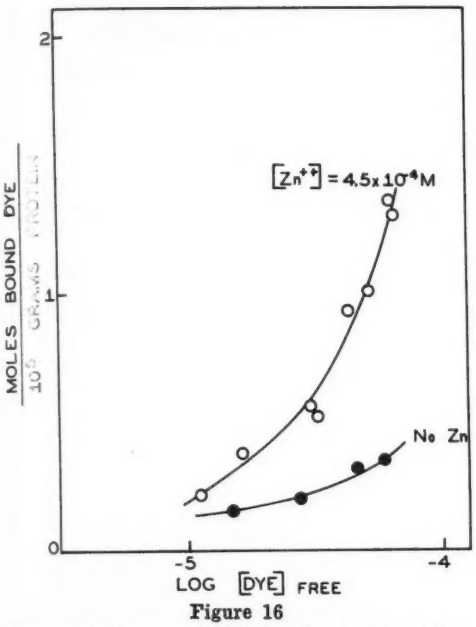


Figure 16

Increase in degree of binding of a dye (pyridine-2-azo-p-dimethylaniline) when Zn^{++} is added to the protein solution. Each curve shows number of bound dye molecules on each protein molecule, as a function of free dye concentration in the solution.

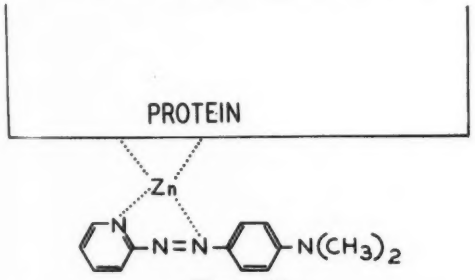


Figure 17

Schematic diagram of Zn^{++} ion acting as a bridge between protein and small molecule. This mediating effect of Zn^{++} increases number of dye molecules bound to protein molecule.

the mechanism of the effect must be markedly different, for glycine itself is not bound by serum albumin. Furthermore, the concentrations of this amino acid which are required to increase the binding of protein[22] are very high (0.5 to 2 M) compared to those at which metals manifest their effects. At such high

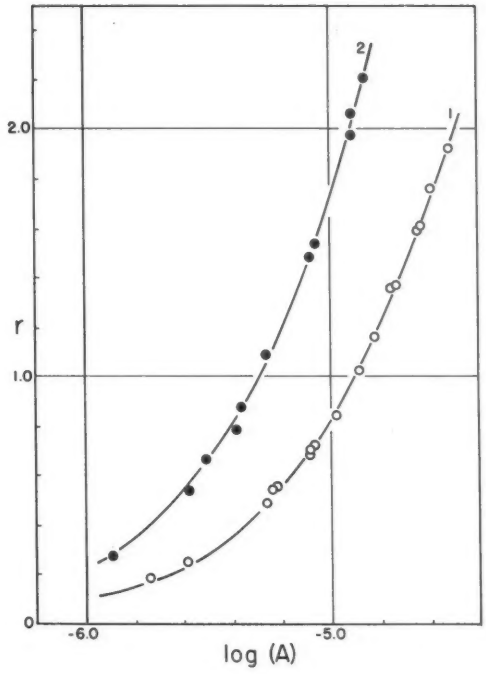


Figure 18

Increased binding of methyl orange by serum albumin when glycine is added to the solution: ○, no glycine; ●, 2.5 M glycine. Each curve shows number of bound dye molecules per protein molecule as a function of the concentration (A) of free dye in solution.

concentrations of amino acid, the dielectric constant of the solution is altered appreciably and, hence, interactions between charged species could be changed. Nevertheless, as we have shown, this electrostatic effect is not the dominant one since even the binding of uncharged molecules by proteins is increased. Instead, it is our feeling, for reasons described elsewhere,[22] that the influence of glycine is to be understood in terms of what this amino acid can do to the solvent rather than to the protein. Zwitterionic glycine perturbs the water in its neighborhood and, hence, also the hydration of the organic molecule in the aqueous solution. Consequently, the organic molecule turns more readily to the more hospitable environment of the protein envelope where, with the assistance of nonpolar side-

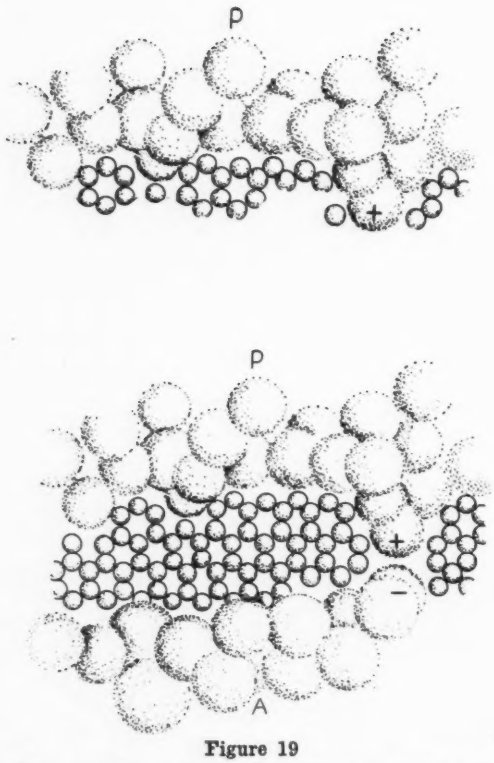


Figure 19

Top: *protein molecule,* P, *with some water of hydration (small circles) around nonpolar regions.* Bottom: *protein complex with an anionic hydrocarbon molecule,* A, *showing increased hydration in the complex due to cooperative effect between* P *and* A.

chains of the macromolecule, a cooperative stabilization of the water lattice may ensue (fig. 19). On this basis, one can see that glycine should affect the binding of uncharged organic molecules as well as of organic ions.

So far, we have considered examples of protein complexes which are essentially reversible (in the thermodynamic sense), that is, which can be dissociated by lowering the concentrations of the reacting species. It should be pointed out that covalently bound complexes may also be formed at physiologic temperatures and pH's. Among the most interesting of these from a physiologic viewpoint are those involving the mercaptan or disulfide substituents of proteins.

As has been mentioned earlier, mercury combines with –SH groups of proteins. An analogous reaction occurs with organic mercurials, e.g., between (II),

(II)

and almost any protein with mercaptan groups:

$$P-SH + RHgX \rightarrow P-S-HgR + HX. \quad (2)$$

The mercaptide bonds formed are very stable; although the rate of the reaction is not always high, the stoichiometry[23] is very sharp (fig. 20). Similarly, sharp stoichiometry is observed in the reaction of disulfide compounds with protein mercaptan groups:[24]

$$P-SH + R-S-S-R \longrightarrow P-S-S-R. \quad (3)$$

Intramacromolecular Interactions of Proteins

It has been known for some time that for many proteins, the kinetic-molecular unit which we see at physiologic pH's and temperatures is not necessarily the smallest molecular species. Under other conditions, the macromolecule may dissociate (or associate) reversibly into smaller (or larger) units. Some of the earliest work in this field is that of Eriksson-Quensel and Svedberg,[25] who demonstrated a number of disaggregation reactions in hemocyanins. A more recent study with β-lactoglobulin,[26–28] is shown in figure 21. The normal molecular weight for this protein is around 35,000. From the shape shown in figure 5, which is based on x-ray investigations, one might not be too surprised to see that this molecule dissociates into 2 equal fragments at low pH. Furthermore, as figure 21 shows, aggregation into units which have molecular weights of 70,000 may also occur at low temperatures, although this is not as complete as the reaction of disaggregation.

In general, disaggregation occurs at pH's far from the isoelectric point, i.e., where the protein acquires a substantial charge. Such behavior is characteristic of many other proteins, for example, chymotrypsin[29] and insulin,[30] as well as of hemocyanin and β-lactoglobulin. With insulin the electrostatic

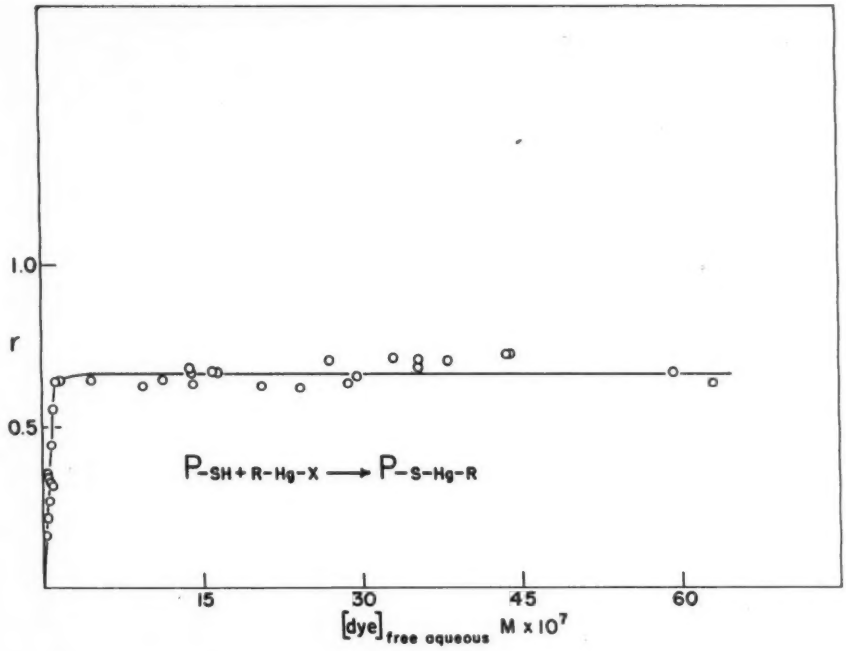


Figure 20

Extent of binding of an azomercurial by serum albumin. The binding rises very sharply even at exceedingly small concentrations of mercurial, and levels off very sharply when combination with all available –SH groups has occurred.

basis of the dissociation has been indicated by further experiments.[30] In acid solution the unit of molecular weight near 36,000 dissociates into particles which have a molecular weight of 12,000 when the charge reaches about +20 to +30 per trimer. In the presence of SCN^- ions, known to be bound to insulin in acid solution, lower pH's must be attained to dissociate the trimer. Obviously, the bound anions reduce the internal electrostatic repulsion in the insulin molecule and hence stabilize the trimer. These observations indicate that many protein molecules are composed of smaller units linked together by relatively weak bonds. The nature of these bonds has still not been established unequivocally.

Intermacromolecular Interactions

In addition to interacting with themselves, protein molecules of a given kind may form complexes with other types of proteins or with other types of macromolecules, including polysaccharides and nucleic acids. One can distinguish in these combinations 2 general categories of complexes: (1) nonspecific, in which electrostatic factors usually predominate; (2) specific, in which distinctive structural factors are of major significance. As an example of each category, we shall cite protein–nucleic acid interactions.

Interactions of proteins with nucleic acids are excellent examples of cases in which opposite electrostatic charge is the primary condition for reaction. Precipitates are formed, for example, between nucleic acid and lysozyme, ovalbumin, serum albumin, fibrinogen, casein, and actin, respectively;[31-33] in all cases the protein is cationic, the nucleic acid, anionic. As we would expect for a complex held together primarily by electrostatic forces, small quantities of salt usually redissolve and dissociate the combination. Soluble complexes may also be formed.[32, 34] In all these cases, the

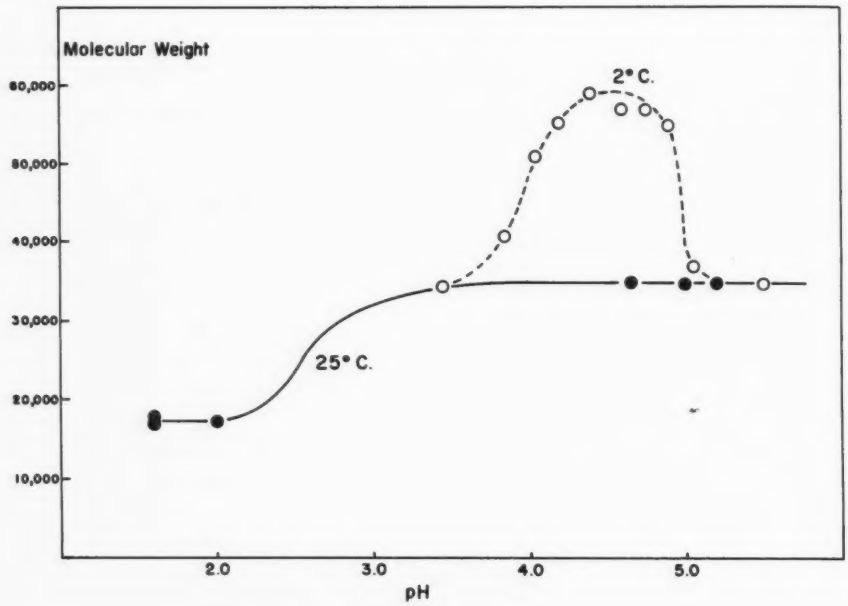


Figure 21

Variations in molecular weight of β-lactoglobulin. Solid line correlates results at 25 C., broken line at 2 C. (Method of presentation taken from Tanford.[28]) The "normal" molecular weight of β-lactoglobulin at its isoelectric point (pH 5) and higher is 35,000. At low temperature and pH's of 4 to 5, the molecules aggregate to dimers. At very low pH, the molecule breaks up into 2 subunits; compare with diagram in figure 5 which is based on x-ray data.

wide spectrum of proteins with which nucleic acid complexes are formed shows the absence of any specificity in these combinations. Theoretical calculations[35] also lead to the conclusion that electrostatic attraction can account quantitatively for the strength of association.

In contrast to these nonspecific complexes, one may cite the highly specific complexes between nucleic acid and protein which are exemplified in the viruses. These combinations lead to special supermolecular structures, with characteristic arrangements of constituent macromolecules. A model for tobacco mosaic virus[36, 36a, 36b] is shown in figure 22. This supermolecule, with a molecular weight of about 40,000,000, has an over-all shape of a long thin rod, 3,000 Å in length and 170 Å in width. It is composed of about 5 per cent ribonucleic acid and 95 per cent protein. The association, however, is hardly the random type found in the complexes described in the preceding paragraph. The nucleic acid seems to be a single strand winding its way through the rod in a helical fashion. In contrast, the protein component seems to be present in subunits which have a molecular weight of about 17,000. These subunits envelop the coiled strand of nucleic acid by winding around it, also in a helical array.

There are other rod-shaped viruses with structures similar to that of tobacco mosaic virus. Many small viruses, however, are quite different in structure, being spherical in shape (for example, turnip yellow virus, fig. 5). Again, interaction between nucleic acid and protein leads to a very specific structure, but with the nucleic acid in the core of the spherical supermolecule anl protein subunits arranged symmetrically in an outer thick shell

Conclusion

Thus, we see that a protein molecule in solution may undergo a host of interactions: with solvent, with small molecules and ions, with itself, with other macromolecules. In a physiologic milieu, all of these possibilities present themselves and will compete among themselves. Reasonable guesses as to which will be the dominant interactions can often be made from behavior observed in some of the simple systems which we have described. It seems reasonable to expect that further studies of such interactions in artificial simple environments may ultimately provide a firm basis for relating the structure of macromolecules to their biologic activity.

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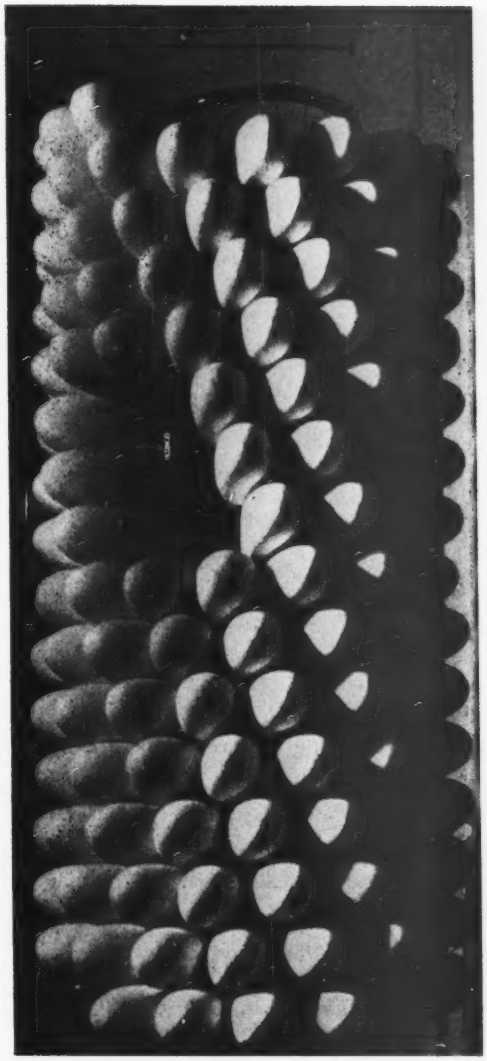

Figure 22

A model of tobacco mosaic virus. The white ellipsoids represent the protein subunits (each about 18,000 molecular weight); the black coil, the ribonucleic acid helix. The protein subunits also wind around in a helical array. The center of the rod is hollow (but filled with water molecules) for a distance of about 20 Å around the axis. (Republished by permission of Endeavour.[38])

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Some Properties of Ionic and Nonionic Semipermeable Membranes

By ALEXANDER MAURO, PH.D.

Some fundamental properties of barriers are reviewed. The barrier of biologic significance is the plasma membrane. The role of the barrier in the transport of solvent and neutral solute species is discussed; 2 fundamental transport processes, namely, diffusion and mass flow are emphasized. The latter is shown to arise as the barrier offers increasing impedance to the movement of a given solute species; the barrier is then referred to as a semipermeable membrane, and the transport of the solvent as osmosis. The osmotic movement, undoubtedly one of the most important transport mechanisms in physiologic systems, is shown to arise from a gradient of hydrostatic pressure in the barrier. The need for the consideration of profiles of thermodynamic quantities is stressed. Finally, the third thermodynamic quantity, electrical potential, is introduced when ionic species are considered in the analysis of the "fixed charge" membrane.

THE objective of physiologists dealing with studies of transport across cell membranes is either to learn what can or cannot pass across the cell surface as one of the basic facts about the cell or, by such data, to obtain some idea about the structure of the membrane itself. It need hardly be emphasized that the main justification for interest in the structure of the membrane is the inextricable relationship between its structure and the mechanisms which operate at this level of the cell to bring about the transport of various species and the associated, higher-order physiologic activity or function, such as propagated activity and secretion. Unfortunately, although we would like to have a clear and definite picture of the membrane phase, our knowledge of its detailed structure has not advanced too dramatically in recent years. With the advent of electron microscopy, the plasma membrane is being visualized as a dark line or a double line of the order of 100 Å in the myelin sheath, muscle fiber, red cell and bacteria. (We should have clearly in mind that many transport studies involve a composite cellular system, such as frog skin, bladder, intestine, colon, capillary and nephron. It is indeed difficult to decide which cells are involved here and, moreover, whether the single cell has a symmetric plasma membrane. Thus, if we desire to keep in mind a simple cell geometry, it would be well to restrict our discussions to single cells of the type seen in muscle and nerve cells, red cells and bacteria. Furthermore, since we shall be concerned here with *some* fundamental physicochemical mechanisms associated with transport, the phenomena of pinocytosis and related topics will be excluded in our discussion.) It is almost a general working hypothesis along the lines suggested by Danielli[1] years ago that the main skeleton of the plasma membrane is a lipoprotein, bimolecular complex that is either "continuous" or "discontinuous" ("holes"). In this bimolecular complex, with the hydrophilic groups extending inward to the cytoplasm and outward to the extracellular fluid, many other molecular entities can be interspersed either radially or along the surface. It should be stated that to date, the main body of evidence for the ultrastructure of some plasma membranes has been acquired by 3 methods of analysis other than electron microscopy, i.e., polarization optics, x-ray diffraction and chemical degradation studies. Most of this work has been done on myelin*

rom the Rockefeller Institute.

*If the Geren theory of myelin formation is correct, then it must be granted that the myelin structure is a multilayered aggregate of plasma membrane. Thus, in the years to come, the combination of x-ray analysis and chemical studies of this system should supply the most detailed "atomistic" information about a plasma membrane that has been found by any preparation hitherto available.

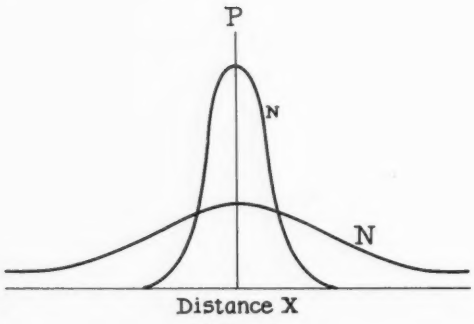

Figure 1

The Gaussian curve showing chance of finding particle a distance x *from starting point for a small and large number of executed steps* n *and* N, *respectively.*

and red cell ghosts, although it would be unfortunate not to mention that a wealth of data has been derived from chemical analysis of bacterial systems.

For our present purpose, let us proceed with the idea that this lipoprotein structure is close-packed and the interstices are filled with water. Those who visualize a predominantly radially-ordered lipid skeleton will picture considerable order in the membrane, and will be inclined toward the notion of "channels" or "pores." On the other hand, the combination of the lipid skeleton and other radially-aligned molecules covered with overlying protein molecules, might conceivably give rise to the idea of a network or meshwork of molecules soaked, so to speak, in water. Whichever picture is preferred the plasma membrane, from this rather glib and simple description, can certainly be considered as some *barrier* to the movement of ionic and nonionic species. Indeed, it is precisely some of the phenomenologic properties which arise in systems acting as barriers that will concern us.

We must now consider what fundamental physical mechanisms can be involved in the transport of various molecular species across the region of the membrane. The most fundamental mechanism which warrants attention is, of course, *diffusion*. Since we must presently consider an alternative mode of transport, which for the present can be referred to as a nondiffusional process, an unambiguous description of the diffusion process is warranted in order to emphasize the difference. Furthermore, by devoting some effort to a more explicit description of diffusion, the vexing and confusing problem of the meaning of "tracer fluxes," "unidirectional fluxes," "influx," "outflux" and "turnover rates" might be seen more clearly.

The most basic kinematic description of the diffusion process was given by Einstein[2] in 1905. This was extended by Smoluchowski in 1915 and most recently by Chandrasekhar in 1942.[3] Einstein's discovery indicated that if a particle executes a "random walk," then a group of similar particles, acting without mutual interference, will distribute themselves in space and time according to the basic diffusion equation

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2}.*$$

Thus, the diffusion equation is accounted for by an explicit description of the displacements of a single particle. Let us examine his arguments: in outline, a particle executes the "random walk"—in 1 dimension—if the a priori probability of jumping a unit step in the forward or backward direction is equal. It is clear that this movement goes on for all time, subject to this condition of equal probability: The *only* question that can be asked further about the particle is the chance of finding it a certain distance in the positive or negative direction from the starting point after a total number of steps have been executed. It turns out that the function describing this probability is the familiar Gaussian curve (fig. 1). It is important to see most clearly what is implied by this function so far as the behavior of the particle is concerned. The function states that starting out at any point, the chance of finding the particle a certain dis-

*In this equation,

$\frac{\partial c}{\partial t}$ = change in concentration per unit time.

D = constant.

$\frac{\partial^2 c}{\partial x^2}$ = the second derivative of concentration with respect to distance.

tance on either side of the starting point is symmetric and the chance "spreads" out the greater the number of steps executed by the particle. Notice this is the only basic description of the behavior of the particle that is possible—it is probabilistic. One might say the particle will wander quite aimlessly on its "Gaussian walk."

It is important to emphasize that thus far we have not introduced any ideas about the energetics involved in the jumping behavior. This will be dealt with later. It is evident that some physical mechanism exists to impart energy to the particles and, thus, to effect an average frequency of displacement. As soon as we begin to consider a distribution of "Gaussian" particles such that at any moment we have a concentration of particles as a function of distance, then, as was shown by Einstein in the paper cited previously,[2] the concentration function will change with time, resulting in a relationship that is expressed by the fundamental Fick equation, usually referred to as Fick's Equation II

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2}.$$

We can invoke another relation which is most fundamental since it expresses the law of conservation of matter, namely, the continuity equation,

$$\frac{\partial c}{\partial t} = - \frac{\partial J}{\partial x},$$

and thus proceed from Fick's Law II to the flux equation (Fick's Law I) known by the familiar term of "Fick's law"

$$J = -D \frac{dc}{dx},$$

where J is the number of particles moving through a unit area per unit time.

As we have stated above, Einstein indicated mathematically how Fick's Law II springs naturally from the movement of many Gaussian particles. However, he also demonstrated a few years later[4] in an "elementary" lecture to chemists—which he was urged to give by Prof. Lorentz—how one could proceed directly from the Gaussian particle consideration to the flux equation, namely, Fick's Law I. Since

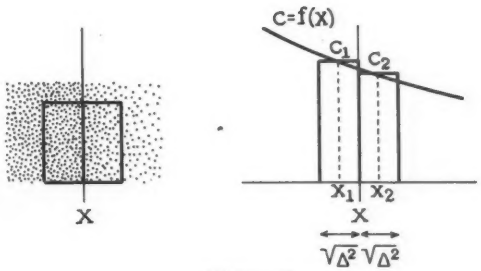

Figure 2

A. Left. *Sketch of the particles distributed with varying concentration. The 2 compartments of equal size are shown on either side of the point* x. B. Right. *Plot of the concentration of particles as a function of the distance* x. *The concentration at the center of each compartment is seen as* c_1 *and* c_2, *i.e., the mean concentration.*

this points up most clearly the fundamental nature of the movements described by the phenomenologic flux equation, it is instructive to reproduce his analysis with some interpolations and comments.

Before proceeding, we must be aware of an important property of the Gaussian particle: if the particle is observed repeatedly at constant intervals of time τ, the arithmetic mean of the squares of the displacements, Δ^2, is equal to $2D\tau$, where D is a constant equal to $\frac{1}{2} nl^2$, n is the jumping frequency and l is the length of the unit step. Let us now choose a region at x in a distribution of particles (fig. 2*A*) whose concentration as a function of x is seen in figure 2*B*, and then confine our attention to 2 rectangular compartments of equal size on either side of x as shown. The width of the elements are chosen to be $\sqrt{\Delta^2}$ cm. The sides are unit area (1 cm.^{2}). Each of the particles contained in either element will suffer an individual displacement, so that a distribution of displacements in time τ will result; the root mean square of this distribution is $\sqrt{\Delta^2}$. Thus, it is clear that for the population of particles in each compartment, one half will suffer a displacement $+\sqrt{\Delta^2}$ and one half $-\sqrt{\Delta^2}$. These conditions are the direct consequence of the fact that the particles are subject to the Gaussian probability function. Since the width of the compartments is

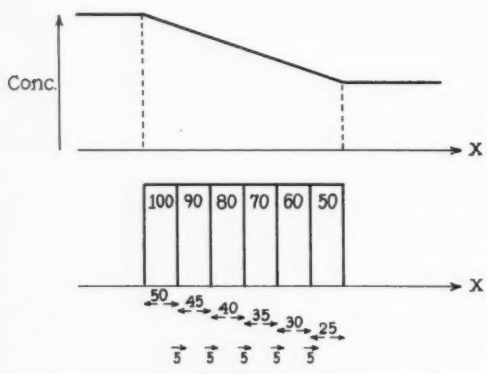

Figure 3

A. Top. *Plot of concentration that varies linearly with distance* x. B. Bottom. *Compartments showing total number of particles moving equally in both directions and the net number from left to right in the interval of time.*

small, it is reasonable to consider the concentrations c_1 and c_2 at the center of each compartment to be the mean concentrations in the respective compartments. Thus the number of particles crossing to the right through the unit area at x is

$$\tfrac{1}{2}\, c_1 \sqrt{\Delta^2}$$

and to the left

$$\tfrac{1}{2}\, c_2 \sqrt{\Delta^2}.$$

The net number is then

$$dN = \tfrac{1}{2} \sqrt{\Delta^2}\, (c_1 - c_2).$$

Since this transfer occurred in a time τ, we have the number of particles per unit time, i.e., the flux is

$$\frac{dN}{dt} = \tfrac{1}{2}\, \frac{\sqrt{\Delta^2}}{\tau} (c_1 - c_2),$$

also we note that

$$\frac{dc}{dx} = -\frac{(c_1 - c_2)}{\sqrt{\Delta^2}}.$$

Thus,

$$\frac{dN}{dt} = -\tfrac{1}{2} \frac{\Delta^2}{\tau} \cdot \frac{(c_1 - c_2)}{\sqrt{\Delta^2}} = -\left(\tfrac{1}{2}\, \frac{\Delta^2}{\tau}\right) \frac{dc}{dx}.$$

If we were to consider smaller and smaller intervals of time, remembering that the term $\tfrac{1}{2}\, \dfrac{\Delta^2}{\tau}$ is a constant, namely, D we then have

$$\frac{dN}{dt} = -D \frac{dc}{dx},$$

the familiar Fick's Equation I. D is known as the "diffusion coefficient" of the particle.

It is instructive to apply this treatment to a distribution of particles whose concentration varies linearly with distance x. The width of the compartments corresponds to a certain interval of time. The number of particles in each compartment satisfies the condition of a linear concentration profile. As explained previously, particles in each compartment will move out in both directions, namely, one half to the left and one half to the right. The net transfer between each compartment is seen to be constant and proceeds from regions of high to regions of low concentration.

This analysis enables us to grasp the essence of the mode of movement of individual particles involved in the process of diffusion. Moreover, we are now in a position to define a nondiffusional process: any movement of particles that involves a component that is nonrandom is thus to be classed as nondiffusional. The pure case, of course, comes to mind in Poiseuille flow, where the gradient of the pressure is associated with laminar flow of particles. The degree of rectilinear flow can, of course, vary so that groups of particles can move along nonrectilinear paths and thus be classed as nonlaminar or turbulent flow.

In general, if a solute is present at a given concentration difference, a diffusion flow of the solute will obtain; concurrently, a diffusion flow of the solvent species will take place in the opposite direction, with a diffusion coefficient equal to that of the solute species. If the solute-solvent diffusion is allowed to take place across a very coarse barrier, e.g., a sintered glass filter, the only effect of such a barrier is to reduce the available cross-sectional area for diffusion.

Thus far in our discussion the barrier has not given rise to any unusual effects worthy of special consideration. However, if the barrier should be modified so that the barrier region begins to offer an impediment to the movement of particles, then a marked increase in the movement of solvent into the solution phase will occur. The maximum flow of solvent occurs when conditions of infinite impedance to the movement of solute particles through the barrier are attained; at this point the barrier is usually characterized as a semi-

permeable membrane, namely, impermeable to the solute but permeable to the solvent. This marked movement of water has been known to biologists for several centuries and has been referred to as osmosis. It is undoubtedly one of the most important effects associated with semipermeability. The question is how should we look upon this phenomenon, i.e., what causes the movement of water—is it a diffusional or a nondiffusional process? It is interesting that both points of view prevail among both physiologists and physical chemists. Since several important points are involved here with regard to the general subject of semipermeable membranes, it is worthwhile to discuss the matter in some detail.

If a collodion membrane is arranged between 2 compartments and means are provided via a capillary tube for viewing the movement of water across the membrane, it is observed that the relationship between the rate of movement of water and the log of the mole fraction of water in 1 of the compartments—the other being pure solvent—is linear. The movement of solvent is seen to occur from the pure solvent to the solution. It is important to emphasize that the collodion barrier is absolutely impermeable to the solute species used to establish the mole fraction of the solvent. If, for a given mole fraction, hydrostatic pressure (in excess of the atmospheric pressure present in both phases) of sufficient magnitude to bring the rate of water movement to zero is applied to the solution, it is found that the magnitude of this pressure is equal to the quantity $(RT/\overline{V})\ \ln N_{H_2O}$ where R is the gas constant, T is the temperature in degrees Kelvin, $\overline{V}$ is the partial molar volume of water and N_{H_2O} is the mole fraction of water. This quantity is known as the "osmotic pressure" of the solution.* In dilute solution in which the N_{H_2O} is close to unity, the expression reduces to its equivalent, RTc, where c is the concentration of the solute species. Repeating the above procedure at a different value of N_{H_2O} establishes the fact that the pressure necessary to reduce the flow of water to zero is always equal to RTc over a wide range of dilute solutions. The equivalence of the mole fraction term, i.e., the "osmotic pressure" of the solution, to the hydrostatic pressure is expressed by the familiar equation:

$$\frac{dn}{dt} = K\ (\Delta P - RTc)$$

where $\frac{dn}{dt}$ is the flow of water per unit time leaving the solution, K is a constant of proportionality, and ΔP is the excess hydrostatic pressure in the solution with respect to pure solvent.

The immediate question then arises: what is the nature of the constant K? This is another way of asking about the nature of the movement of the solvent across the membrane when it is acted upon either by a hydrostatic pressure or by a difference in the mole fractions of the solvent across the membrane. The most direct way of establishing whether K is a diffusion-permeability coefficient for the barrier under investigation is to apply the more fundamental form of the diffusion equation; as indicated by the theory of irreversible thermodynamics, this equation should be expressed in terms of the gradient of the chemical potential,[5, 6] namely,

$$\frac{dn}{dt} = \frac{DA}{RT}\, c\, \frac{d\mu}{dx} \qquad (1)$$

where D is the diffusion coefficient of the solvent, A the area of the available "pores," c the concentration, and μ the chemical potential. We shall make the reasonable assumption that the membrane is "uniform" and thus that the applied ΔP gives rise to a linear gradient, namely $\Delta P/\Delta x$ is a constant. Remembering that only pure solvent is present in the membrane, the chemical potential is simply a function of pressure, thus,

$$\Delta\mu = \overline{V}\Delta P \text{ and } \frac{\Delta\mu}{\Delta x} = \overline{V}\,\frac{\Delta P}{\Delta x}$$

and finally,

$$\frac{dn}{dt} = \frac{DA}{RT}\, c\overline{V}\,\frac{\Delta P}{\Delta x} = \frac{1}{RT}\,\frac{DA}{\Delta x}\cdot \Delta P \qquad (2)$$

since $c\overline{V} = 1$. The diffusion permeability co-

*Two other parameters related to the "osmotic pressure" are "lowering of vapor pressure" and "lowering of freezing point." All 3 are related to the mole fraction of the solvent.

Table 1

Flux in moles/sec. per dyne/cm.²

Membrane	Diffusion flux[4]	Total observed flux	Ratio diffusion /total
Group I	1.2×10^{-13}	8.8×10^{-11}	1/730
Group II	5.6×10^{-14}	3.1×10^{-12}	1/55
Group III	1.1×10^{-14}	3.9×10^{-13}	1/36

efficient DA/Δx can be evaluated by performing a tracer experiment with H_2O^{18} subject to the familiar form of Fick's equation, namely,

$$\left(\frac{dn}{dt}\right)_{H_2O^{18}} = \frac{DA}{\Delta x}(\Delta c)_{H_2O^{18}}.$$

By sampling the 2 compartments at suitable intervals of time and by using the mass spectrometer to determine the concentrations of H_2O^{18} present, the integrated form of the above equation (see [8]) can be invoked to calculate DA/Δx of the membrane. Having established this parameter, we can then estimate the flow of water which moves by diffusion across the barrier when a pressure is applied by means of equation (2). Data of this kind[7, 8] are seen in table 1 for 3 groups of collodion membranes (see also fig. 4). The membranes are essentially of the same thickness but have acquired different "pore" sizes for aqueous flow by suitably modifying the procedure used to prepare the membranes.

The first fact shown by the data is that, in all 3 groups, the diffusion component, due to a gradient of chemical potential resulting from the pressure gradient, is small with respect to the total flux; therefore, the flow of solvent must have the character of a nondiffusional flow. Many authors[9-12] have considered that the flow obeys Poiseuille's law, and have imagined the barrier to consist of uniform cylindrical pores. These authors have then proceeded, using comparable data obtained on biologic cells,* to calculate an average "pore" diameter. The second fact is the convergence of the nondiffusional and diffusional component when "coarse" membranes are compared (group I) with the "tight" membranes (group III). The pertinent data appear in column 4 of table 1. This convergence supports the concept of Poiseuille flow through pores,[8] but some reservations should be held about the validity of using the Poiseuille conductance for flow in "pores" of very small dimensions.

*In biologic studies, the movement of water is usually induced by osmosis since it is virtually impossible in many cases to apply a ΔP across the cell surface.

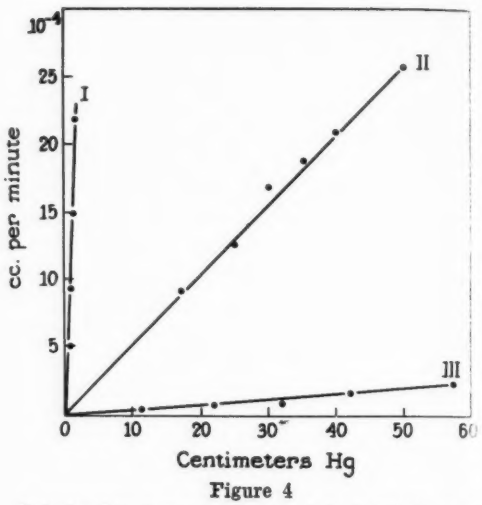

Figure 4

Relationship between flow and hydrostatic pressure for 3 groups of membranes. Note flux in cc./min. per cm. Hg can be converted to moles/sec. per dyne/cm.² by multiplying by .7 × 10⁻⁷. (Republished by permission of the Journal of General Physiology.[8])

At this point, it is pertinent to consider osmotic flow once again, i.e., the flow associated with the presence of an impermeant solute which serves to establish the mole fraction of the solvent at less than unity. Since the previously described "osmometer" experiment has demonstrated that the mole fraction term, i.e., the "osmotic pressure" is exactly equivalent to the hydrostatic pressure, it follows that the mode of transport of water during osmosis and during the application of a pressure must be identical. Consequently, our previous discussion indicates that this flow must be nondiffusional. A further consideration of this matter will now be used to cast some light on the origin of osmotic flow and to stress the general importance of consider

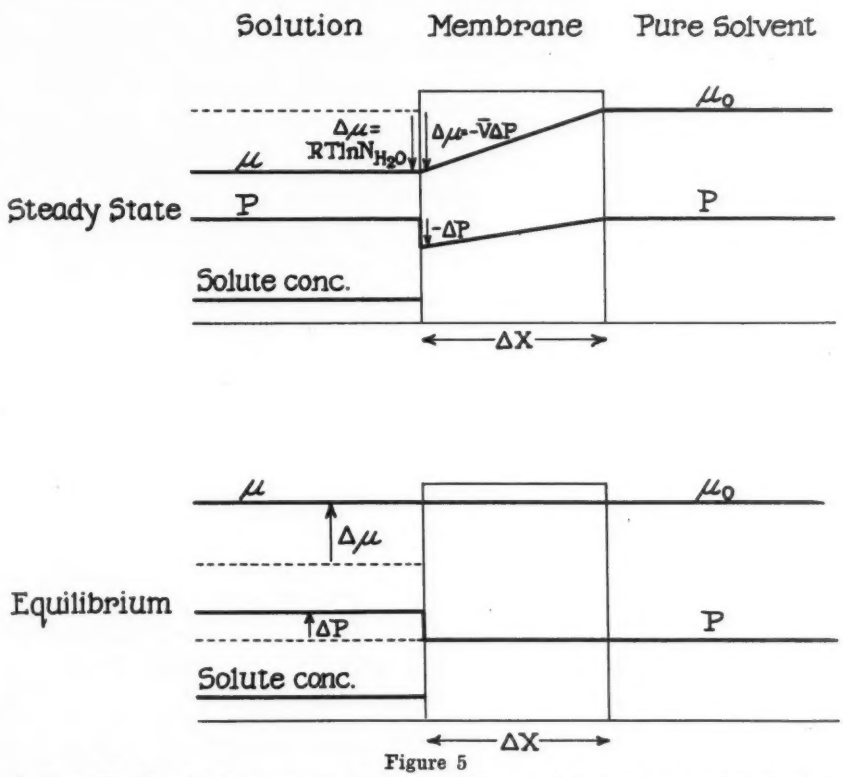

Figure 5

A. Top. *Profile of solute concentration, chemical potential of solvent and hydrostatic pressure for the steady state.* B. Bottom. *Profile of the same system for the condition of equilibrium brought about by applying ΔP to the solution.*

ing the "profile" of thermodynamic quantities within barriers.

With the aid of figure 5, we can see the profile of the chemical potential of the solvent in the solution phase. Note that the decrement in chemical potential $\Delta\mu$ is equal to $RT\ln N_{H_2O}$ where a value of N_{H_2O} different from unity has been established by the presence of the *absolutely* impermeant solute. It is apparent from a consideration of the interface between the solution and the barrier that just inside the barrier, where there is only pure solvent, the continuity of the chemical potential function can only be satisfied by another component of the chemical potential, i.e., $\overline{V}\Delta P$. Thus, on the solution side of the interface the decrement in chemical potential is $RT\ln N_{H_2O}$ and, in the barrier, $-\overline{V}\Delta P$. It follows, then, that a drop of pressure, $-\Delta P$, must exist at the interface of the barrier and the solution, and thus serves to act as the "driving force" for the osmotic flow. The pressure profile which results is seen in figure 5*A*. Note that the condition of thermodynamic equilibrium is attained when a hydrostatic pressure, ΔP, which is clearly

$$(RT/\overline{V})\ln N_{H_2O}$$

is applied to the solution. Such a maneuver is actually carried out with the osmometer to establish the thermodynamic parameter of the solution, $(RT/\overline{V})\ln N_{H_2O}$, which for dilute solutions reduces to its equivalent RTc. The profile of chemical potential for the conditions of equilibrium is seen in figure 5*B*.

The argument pursued here is essentially

similar to that advanced by Garby in 1957[13] and might be classed as a thermodynamic approach. Unfortunately, there is lacking at present an explicit kinetic theory which explains the origin of the pressure drop, and thus the osmotic flow. Incidentally, the explicit treatment of the pressure profile in a barrier seems to have been carried out for the first time by Schlögl in 1955[14] in an attempt to explain the origin of anomalous positive and negative osmosis in ionic membranes. We shall not pursue this matter further except to emphasize the importance of recognizing, in general, the existence of the hydrostatic pressure throughout the membrane phase, especially for the understanding of *ordinary* osmosis.

To underline the role of the pressure gradient which arises for the condition of absolute semipermeability, it is instructive to deal with the case when 2 solutions are present, one containing an absolutely impermeant species and the other a permeant species.

However, before proceeding, it should be stated that if the "osmometer" experiment were performed with a "leaky" solute, the effect observed with a given RTc would be less than the corresponding ΔP. This is the other striking property of the osmotic mechanism which can be succinctly expressed by writing the osmometer equation given above in its more general form,

$$\frac{dn}{dt} = K\ (\Delta P - \sigma RTc)$$

where σ is a constant—referred to by Staverman[15] as the "reflection coefficient"—that takes on a value approaching zero for very permeant species and unity for absolutely impermeant species.

With this in mind we can now consider the behavior of the case in which 2 solutions interact with a given barrier. It is clear that the permeant species gives rise to a negligible pressure drop. Thus, within the barrier, the gradient of pressure which is due to the presence of the impermeant species in the other solution is a "driving force" *common* to all species in the membrane. This common "driving force" imparts a mass flow to both solvent and permeant solute. Simple and elegant experiments demonstrating this effect have been conducted by Meschia and Setnikar[16] on a collodion membrane which has the characteristics of group I cited previously. Unawareness of the pressure drop in the barrier would prompt one to predict that solutions of identical mole fraction, e.g., dextran vs. urea, would give rise to zero movement of water, especially if the customary use of the chemical potential of the solvent in either phase is used in a "discontinuous" treatment of the barrier. Although this matter will not be pursued in detail, it will suffice to state that a "discontinuous" treatment of a barrier must be a "complete" treatment involving chemical potentials of all species, as carried out by Kedem and Katchalsky.[17] Otherwise, an attempt can be made at a "continuous" approach such as we have outlined above in order to establish the thermodynamic profiles within the barrier and thus to predict the movement of the various species.

Thus far in our discussion we have been dealing with the characteristics of semipermeable barriers that arise primarily because the meshwork or network of "porous" material constituting the membrane presents a simple mechanical hindrance to the solute species. However, it is possible to observe a broad class of membrane activity which gives rise to selective ionic permeability and electromotive forces in addition to osmotic effects *without* involving direct mechanical blocking of ionic movements. The kernel of this new mechanism is the Donnan effect. The most direct way to appreciate this mechanism is to imagine a gel made up of macroions, such as protein or other polyelectrolyte, and, of course, of appropriate mobile ions of the same sign ("coions") and of opposite sign ("counterions"). If the gel block is arranged to separate 2 solutions containing coions and counterions at the same concentration, the gel phase and the 2 solutions will enter into a "symmetric" Donnan equilibrium. One of the important consequences of this state is that ions of the same sign as the macroions are present at low concentration approaching

vanishingly small magnitude as the concentration of macroions is increased. Without entering into a detailed thermodynamic treatment of the Donnan system, it will suffice to state that all mobile ions tend to be distributed so that the total chemical potential of each species is a constant over the entire geometry. Disregarding for the moment the role of the pressure component of the chemical potential, we must now consider another important component, the electrical potential. Analysis indicates that an electrical potential variation or profile must exist throughout the interface of the gel and the solution arising from a statistical diffuse "double layer."[18] In fact, it is the interplay of this profile of electrical energy and the concentration component of the chemical potential that brings about the equilibrium condition. If the solutions have different concentrations, the state now is "asymmetric," that is, the 2 gel-solution interface regions remain very close to the Donnan equilibrium, but throughout the gel a diffusion regime of ions is established. (Profiles of potential and concentration can be seen in figures 6 and 7; these profiles were[19] obtained from a realistic model.) If the macroion concentration is very large with respect to the ions in either solution, the "tilt" of the concentration profile throughout the gel is minimal, and thus diffusion is minimal. The gel in this case is referred to as being semipermeable to the electrolyte, but selectively permeable to the counterions present in the gel. If the polyelectrolyte is associated with a 3-dimensional network of polymers to which ionogenic groups are attached by chemical forces, this matrix as a whole will display exactly the same properties as the gel and is referred to as an ionic membrane, or, to emphasize the immobility of the ionogenic groups, as a fixed-charge membrane. The theory of such a barrier as we have outlined is contained in the papers of Teorell[20] and Meyer and Sievers. The more complete treatment which takes into account the role of pressure profiles as well as the concentration and the potential has been carried out by Schlögl.[14] It should be emphasized that by increasing the density of

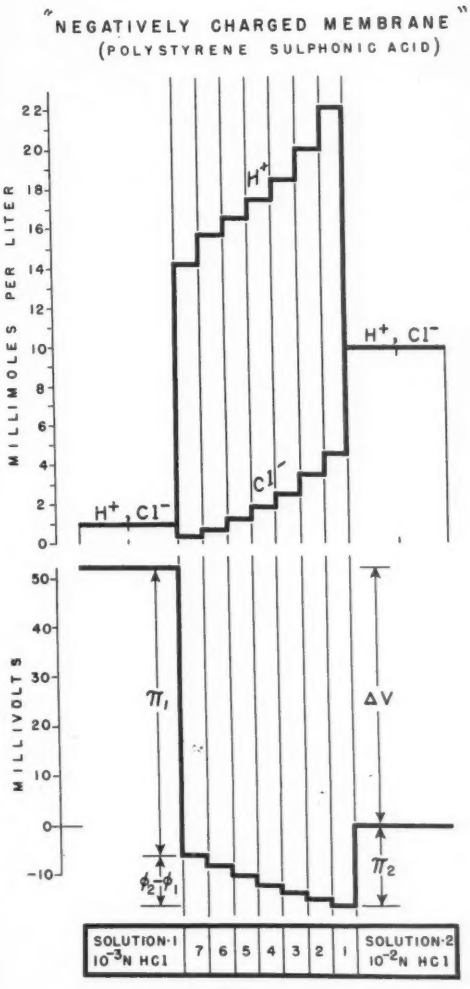

Figure 6

Model of ionic membrane constructed by use of multicompartment cell. Polystyrene sulfonic acid provides negatively charged macroions. The profiles of concentration and potential are seen throughout the "membrane." (Republished by permission of the Journal of General Physiology.[19])

cross-linking, the matrix will eventually display, in combination with the Donnan effect, the properties of mechanical hindrance to solute species, suggesting to many investigators the concepts of "pores" and "channels."

This brief treatment of some properties of "inert" membranes, both ionic and nonionic,

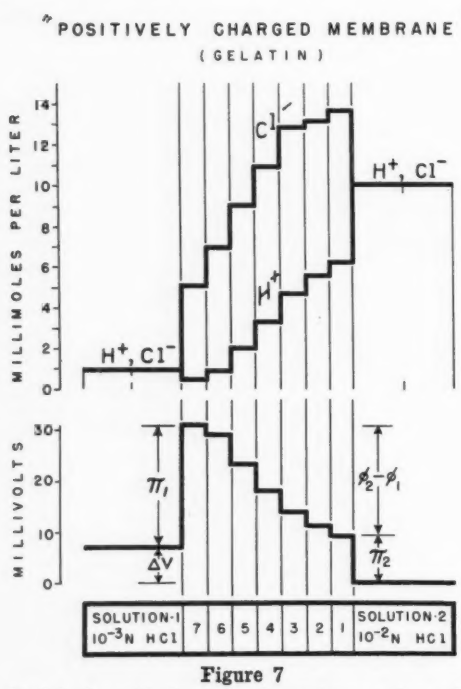

Figure 7

Model of positively charged membrane using gelatin to provide positive macroion. (Republished by permission of the Journal of General Physiology.[19])

should serve to illustrate the interrelationship between various physical chemical parameters in relatively simple systems and perhaps to shed, though feebly, some light on the cauldron of activity in physiologic membranes.

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Discussion

Dr. Wheeler: I should like to ask Dr. Klotz, in relation to the binding of substantial amounts of water by protein, whether considerable quantities of protein in solution may, in effect, by removing water from solution, increase the relative activity of other constituents in the solution?

Dr. Klotz: I think that seems like a reasonable proposal. A similar thing happens even in highly concentrated solutions of electrolytes.

Chairman Smith: Dr. Klotz, would you call that "bound" water?

Dr. Klotz: You can call it what you like. "Bound water" is a name, but not a definition of what you are measuring. You will get different answers from different people I am sure.

Chairman Smith: "Bound water" is an historical enigma of biochemistry and biology.

I should like to cast back in history, for just a moment, on the question of the nature of semipermeable membranes in Dr. Mauro's presentation. You remember, the way in which Pfeffer made his osmometer was to diffuse ferrocyanide and copper salts through the porous wall of an unglazed earthen pot, in order to precipitate a membrane of copper ferrocyanide in the wall. This membrane was relatively thin. Osmometers as later prepared by Morse and his colleagues at Hopkins, when used with sucrose, withstood an osmotic pressure of 12 atmospheres (176 pounds per square inch) for 60 days, without leaking detectable quantities of sucrose and without undergoing detectable diminution in osmotic pressure. The osmometers prepared by Lord Berkeley and his colleagues supported pressures up to 250 atmospheres (3,575 pounds per square inch) for shorter periods. To my mind, this is close enough to an ideal membrane, in a sense, to do business.

But with respect to the nature of the membrane and the theory of "random walking," the Abbé Nollet's membrane of pig bladder was permeable to water, but, paradoxically, not to alcohol which is more volatile than water. Some animal membranes, if first soaked in water, are permeable to ether but not to water, although presumably wet by water.

Water, but not salt, can osmose through a thin slab of marble. A rubber membrane is permeable to pyridine but not to water or sucrose, both of which are quite soluble in pyridine. A good copper ferrocyanide membrane is impermeable to sucrose, potassium chloride, and lithium chloride, but quite permeable to ethylamine hydrochloride and relatively permeable to sucrose.

When Einstein proposed a probabilistic treatment in terms of what has been called a "random walk," he erected, did he not, Dr. Mauro, a postulate? In the examples that I have cited, we have a series of paradoxes, presented by relatively simple semipermeable membranes, not as complicated as the plasma membrane. I wonder if Dr. Mauro would care to comment upon the paradoxes presented by the membranes which I have mentioned, and give us something that is molecularistic as well as probabilistic, specifically, how "random walk" occurs across membranes with highly selective permeability.

Dr. Mauro: I am a little confused about the question, Dr. Smith.

Chairman Smith: I think I am asking how far the notion of "random walk" can be useful in describing the relatively simple membranes that I mentioned.

Dr. Mauro: A way that one can answer this question is simply to state: the general condition that has to be established in order to predict how a given species will get across the barrier is to establish the thermodynamic gradients that are present. In the simplest types of membrane—in those that I discussed, the collodion membranes—we found that the movement involves only 1 species, the solvent, i.e., water. There is no question about this. The movement cannot be predicted, nor can it be accounted for by "random walk," that is, by a diffusion process. Therefore, one must

conclude that it is some other process, namely, a nondiffusional one.

As we pursued the matter further, we became convinced, on thermodynamic grounds, that there must be a pressure drop in the barrier; consequently, within the barrier the thermodynamic driving force is the gradient of pressure which operates on the solvent molecules to drive them across. If the barrier is very, very tight, getting into the highly "ordered" region at which Dr. Podolsky hinted, namely, so-called "pores" that are probably 1 or 2 water molecules in diameter, the only possible way that water could move across would be by a diffusion process.

Chairman Smith: For the clarification of some of us, historically, van't Hoff began with a difference in vapor pressure, and I think historically we can ignore the fact that vapor pressure is defined relative to the vapor phase. The meaning of the term is clear, whether or not we are dealing with a semipermeable membrane. You switched this morning to Willard Gibbs' chemical potential. Is anything gained thereby or is there anything significantly different?

Dr. Mauro: Yes, there is, Dr. Smith. The chemical potential is a much more basic parameter. For one thing, it is much more basic from the point of view of the general theory of thermodynamics and from the point of view of any kind of statistical mechanical calculation which you do on a real system. The chemical potential describes the system more basically from the energy point of view. Vapor pressure is one of the consequences of differences in chemical potentials, so to speak.

Dr. Podolsky: Could I just add something to Dr. Mauro's remarks? You can't very well speak of vapor pressure of crystalline sodium chloride, or even of sodium chloride in water. I think that this deficiency is a clear demonstration of where chemical potential might be more useful.

Chairman Smith: It was so spoken of historically when the colligative properties of freezing point and vapor pressure were introduced.

Dr. Podolsky: Well, speaking of theft, as you did before, that practice is one order of magnitude greater. (Laughter)

Chairman Smith: You have answered my question. I think Dr. Mauro also abandoned—correct me on this if I am wrong—the concept of diffusion per se, even in the ferrocyanide membrane, in favor of Poiseuille flow. Am I correct?

Dr. Podolsky: Oh, no, I wouldn't say so.

Chairman Smith: If I am wrong, please correct me.

Dr. Mauro: I do not know what the hydraulic conductivities of the ferrocyanide membranes are. I should like to point out that as the membranes become very, very "tight," then it is a rather academic question to argue whether the water is moving across on molecular-molecular basis, by a Poiseuille flow, or by a "random walk" process.

However, in a relatively "coarse" membrane, such as those with which we dealt experimentally and from which the data that you saw on the screen were obtained, the nondiffusional flow is clear-cut. Even in group III, for example, in which those barriers begin to get rather tight, as indicated by the fact that they show osmotic effects of about 20 per cent for a glucose solution, the diffusional component has been calculated to be rather small. If you had a so-called solid membrane, the type that Laidler and Shuler thought they were analyzing, the difference in the vapor pressure or in the chemical potential would be related simply to the flux through some kind of diffusion coefficient for the barrier. In general, for an absolutely impermeant solute, the movement of water is proportional to the chemical potential difference or to the vapor-pressure difference; however, the rate at which water moves across is such that the nature of the proportionality coefficient is not a diffusion coefficient. I tried to point out that there is something quite different here which is really the essence of the osmotic mechanism: a gradient of pressure develops within the barrier which drives the water across.

The other way to emphasize the importance of this pressure is to point out that if you

bathe that same barrier with 2 solutions of identical vapor pressure, that is, of the same mole fraction, depending upon whether the vapor-pressure lowering is brought about with an impermeant species or a permeant species, you get entirely different movements of water across the barrier. The most dramatic way to see this in the same simple membrane is to carry out an experiment with a given concentration of dextran and the same concentration of urea. If you do such an experiment, you find that the urea solution moves across at a rapid rate, quite oblivious to the fact that its mole fraction is identical with that of the dextran.

In such experiments, epitomized by the very elegant and simple experiments of Drs. Meschia and Setnikar, the difference in the vapor pressure across the barrier—vapor pressure or any of the other colligative properties —will *not* tell you unambiguously what happens to the movement from 1 compartment to the other. You have to analyze the problem. You have to dissect the membrane, if you want to use this "continuous" thermodynamic figure that I used (rather crudely, I admit). If you want to be more formal, the so-called "discontinuous" treatment can be used as pioneered by Staverman and by Kedem and Katchalsky in their paper in the *Biochimica et Biophysica Acta*. Their paper is certainly a very impressive first attempt; although there are several points that one might criticize, they do show that in order to predict the general movement of either solute or solvent across the barrier, you have to invoke the general chemical potential equation, and the so-called "irreversible" thermodynamic theory of Onsager. Here again we see that the permeant species does not exert the full osmotic effect.

Chairman Smith: Granted, you have to know your semipermeable membrane before you talk about it, but, knowing that your semipermeable membrane is permeable to urea, I would come back again to the challenge of whether you gain anything in terms of chemical potential as compared with the classical treatment.

Dr. Berliner: I just want to know if Dr. Mauro would like to place biological membranes in the spectrum of membranes of various hydraulic conductivities about which he spoke.

Dr. Mauro: I think, Dr. Berliner, as you know, that there are some relevant data. I should have mentioned that, over the last 7 or 8 years many physiologists have been performing the same calculations as those which I tried to outline on the collodion membrane. Dr. Pappenheimer and Dr. Ussing, as a matter of fact, have pioneered this type of analysis; Dr. Nevis has also done it on lobster and squid axons. Incidentally, it is quite universal in most biologic systems that one brings about the osmotic flow, the Poiseuille flow or quasi-Poiseuille flow, not by applying pressure across the plasma membrane—because that is rather difficult to do in many cases except, perhaps, the frog's skin or the gastric mucosa —but by the osmotic effect; namely, by using a solute that can't get across to perturb the plasma membrane osmotically. In such an experiment you find, with rare exception, as perhaps in the trout egg, that the hydraulic conductivity is always greater, i.e., the hydrodynamic permeability or the osmotic permeability is always greater than the diffusion permeability. Therefore, in most cells, the osmotic flow is predominantly a nondiffusional flow.

Chairman Smith: Consider something "simple" like the plasma membrane and work backward, as Ostwald said, seeking a knowledge of the basic nature of the pure liquid. Would you consider, Dr. Mauro, Dr. Podolsky, Dr. Klotz, that the plasma membrane in any of its parts should be treated in terms of the semisolid state?

Dr. Mauro: I'm sorry, Dr. Smith, that I didn't emphasize this aspect in my introductory remarks. There is no question but that the plasma membrane should be looked upon in terms of Dr. Podolsky's notion, as highly "ordered" structure, so that movements of molecules and ions are not just going across broad spaces of water. They simply have to fit into an ordered system. We will probably

know more about this in years to come, when we learn more about the actual structure of the plasma membrane.

As I indicated, the most promising line of attack seems to be the x-ray and diffraction studies, such as are being carried out on myelin by Dr. Finian in England. The myelin sheath appears to be a multilayered membrane of the Schwann cell. Aside from an intellectual attack, where one tries to predict microscopic data from macroscopic observations, x-ray diffraction is the only way to get at the problem of securing ultra-structural information. If we continue this type of study on the myelin sheath, we will probably learn something about the real ultra-ultra structure of the plasma membrane and just how highly ordered it really is.

Chairman Smith: Well, gentlemen, our time is up. I will close by telling a story, and I hope Dr. Szent-Gyorgyi is here while I tell it. Dr. Otto Loewi's office is across the hall from mine. One day he stopped me and put his arm around my shoulder and said, "Homer, it's all in the plasma membrane, it's all in the plasma membrane." I said, "Yes?" He said, "What is all this stuff inside the cell for anyway? I'll tell you a story. Dr. Szent-Gyorgyi was speaking at a Woods Hole seminar and he repeatedly referred to the plasma membrane, and some courageous soul in the audience held up his hand and said, 'But, Dr. Szent-Gyorgyi, would you define the plasma membrane for us, or draw a picture on the board or tell us what its structure is?' Dr. Szent-Gyorgyi paused and thought a moment and said, 'No, the plasma membrane is like God. Everybody talks about it but nobody has seen it.'" (Laughter)

I hope, 10 years from now, we will know something of the ultra-ultra structure of the plasma membrane.

Dr. Mauro: May I make a comment on that, Dr. Smith? I think I must class you in that group of physiologists whom, you remember, I mentioned at the beginning of my talk as those I was trying to discourage.

I simply want to say that it is quite true that many of the phenomena of physiology are being rather glibly ascribed to the so-called plasma membrane. It would really be very unfortunate if we went away thinking that there are many phenomena in cellular physiology that have to be associated solely with the membrane.

My main line of interest in the plasma membrane is in connection with excitable tissue. I can assure you that in excitable tissue there is a wealth of data to convince you, without having any kind of sophistication, that this membrane constitutes an organized stratum. For example, if you simply put a big "sewer pipe" electrode in the squid axon, you can move it around with impunity inside the axoplasm for about 300 or 400 or 500 μ as long as you do not scrape the inner surface; once you do, you can go out for a swim for the rest of the day because the axon is "gone." There is no question that there is a highly organized structure at the surface. While it is quite true that we don't know too much about this structure, there is no doubt that it is some kind of ordered lipoprotein system such as that which we know from the studies on the red cell ghost and the myelin sheath.

I think that the skeptical point of view has even crept into the electrophysiological literature where, within the last 5 years, there has been a rather charming kind of supercritical remark about the plasma membrane in excitable cells. This has taken the form of "Well, it's simply an interface phenomenon or simply a line of discontinuity between axoplasm or cytoplasm and extracellular fluid." This would be quite wrong and quite incorrect.

Chairman Smith: Dr. Mauro, I assure you that even if you put it in quotes, I still believe in the plasma membrane. Now, gentlemen, we are a little over our time and we must adjourn.

II. INTRARENAL SITES OF SALT AND WATER EXCHANGE

Chairman: Robert F. Pitts, Ph.D., M.D.

INTRODUCTION

By Robert F. Pitts, Ph.D., M.D.

In all vertebrates except, perhaps, the marine fishes and birds, total body content and concentration of salt and water are regulated primarily by the kidneys. Precision of regulation and the ability to meet the stresses of deprivation or of gross excess of intake are dependent on osmoreceptor–antidiuretic hormone and volume receptor–adrenocortical mechanisms of control. Our topic this afternoon is "Intrarenal Sites and Mechanisms of Salt and Water Exchange." Extrarenal regulatory mechanisms will be considered tomorrow.

Over the past 30 years or more, an extensive body of knowledge has accumulated describing the gross over-all operation of these renal mechanisms. We are at present entering an exciting phase of increasing understanding of the renal processes at a cellular level. The foundation for this understanding was laid nearly 30 years ago, by Dr. A. N. Richards and his associates in their pioneering studies with micropuncture technics on the amphibian, and later, on the mammalian kidney.

Further development of knowledge and the testing of hypotheses on intact animals and man depended in major part on the clearance methods and concepts developed largely by Dr. Homer Smith. And yet, despite a mass of descriptive information, there has been no very satisfactory understanding of mechanisms of excretion or of reabsorption of salt and water at a cellular level.

I propose that the major developments of understanding which occur periodically at some 10- to 15-year intervals depend on 3 factors: first, the gradual accumulation of a critical mass of basic information; second, the new concept or way of viewing the problem; and third, the new method, or adaptation of the old, which permits a more precise testing of the concept.

Our first topic this afternoon is "Osmotic Concentration and Dilution in the Mammalian Nephron." Ten to 15 years ago, our understanding was reasonable, though not profound. In water deprivation, antidiuretic hormone stimulated the active pumping of water in the distal convoluted portion of the nephron. Solutes remaining in the tubule were concentrated by the extraction of water. A concentrated urine of small volume was formed. Then, it became evident from micropuncture studies that the urine within the distal tubule is hypo- or isotonic even when the final urine is markedly hypertonic. The pumping of water was shifted to the collecting ducts.

Some began to grumble about the pumping of water. Can any cell transport water actively? Based on this and far more background information, a new concept originated with the chemists, Hargitay and Kuhn, and the physiologist, Wirz; ions were pumped actively by the loops of Henle to establish an osmotic gradient between cortex and medulla. Water diffused passively down this osmotic gradient as urine flowed along the collecting ducts. No pumping of water was necessary.

At first, little credence was accorded this concept in this country. Perhaps it was not

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clearly explained. Our first 2 speakers, Drs. Gottschalk and Ullrich have contributed significantly to this thesis of osmotic concentration and dilution of the urine. They have used different approaches. Dr. Gottschalk, with a background of experience in micropuncture technics obtained in a study of intratubular and peritubular capillary pressures, has provided impressive data in support of this view of formation of a concentrated urine. Dr. Ullrich, working with ureteral microcatheterization in the hamster, has done much to clarify the role of the collecting ducts in forming a concentrated and a dilute urine.

The second part of the program this afternoon deals more specifically with the mechanisms of transport of ions, although there will be continuing reference to the transport of water as well. The first speaker, Dr. Giebisch, could qualify as the grandson, scientifically speaking, of Dr. A. N. Richards—*if* Dr. Phyllis Bott will permit me to call her his mother. (Laughter) The reason why I say this is that Dr. Giebisch learned the technic of micropuncture through the kindness of Phyllis Bott who took him into her laboratory and devoted time to his training. His presentation will be concerned with the measurements of electrical potentials and ion fluxes in single nephrons. The second speaker, Dr. Berliner, is really a perfect example of one who needs no introduction. His basic and fundamental work, not only on ion exchange but also on other aspects of renal function, are well known to all of you. He will deal with mechanisms involved in ion exchange in the nephron. The final paper on this afternoon's program is by Drs. Malvin and Wilde. They will consider the role of "stop-flow" studies in elucidating ion and water reabsorption in the nephron of the dog. As many of you know, Dr. Malvin and Dr. Wilde have pioneered in a new method of study of renal tubular functions, a sort of a poor man's micropuncture technic, as I like to look upon it. I say this in no derogatory sense, since I am a poor man myself and have used their method extensively. At the close of these papers, the participants will have the opportunity to appraise the information and concepts which have been derived from these diverse sources.

Osmotic Concentration and Dilution in the Mammalian Nephron

By CARL WILLIAM GOTTSCHALK, M.D.

The micropuncture evidence relating to the location and the mechanism for concentration and dilution of the urine is reviewed. As required by the countercurrent hypothesis for urine concentration, these data demonstrate that in the presence of antidiuretic hormone, the tubular urine is first concentrated in the descending limb of the loop of Henle and then diluted in the ascending limb of the loop before its final concentration in the collecting ducts. The loop of Henle is believed to function as a countercurrent multiplier system, and the vasa recta as countercurrent diffusion exchangers. Additional data are required during water diuresis before the course of events in this condition is established.

THE micropuncture technic which was originally applied to the study of kidney function by Wearn and Richards[1] has been particularly fruitful in elucidating the course of events as they occur in concentration and dilution of the urine. Over 20 years ago Walker and his collaborators[2] demonstrated that proximal tubular fluid in frogs and Necturi was isosmotic with plasma, and that the hypo-osmotic urine characteristic of these animals first appeared in the distal tubule. In 1941 Walker, Bott, Oliver and MacDowell[3] demonstrated that a large percentage of the glomerular filtrate was reabsorbed proximally* by an isosmotic process in rats, guinea pigs, and 1 opossum. They were able to collect only 3 samples of fluid from distal convolutions. One of these was isosmotic, but the other 2 were hypo-osmotic. This latter observation was rather surprising, as the final urine was hyperosmotic. They were reluctant to attach too much significance to their few analyses of distal fluid, but pointed out that the findings suggested that the site of active chloride reabsorption was proximal to that of pure water.

More recently, Wirz[4] reported that proximal tubular fluid was also isosmotic in rats undergoing a water diuresis, and that fluid in the early distal convolution was hypo-osmotic under all conditions. During water diuresis, the fluid remained hypo-osmotic throughout the distal convolution and collecting ducts, but became isosmotic by the end of the distal convolution when the final urine was hyperosmotic. Miss Mylle and I have been able to confirm most of the above results, and to extend them.[5] This is fortunate, for the 3 groups have worked independently and have been widely separated, either by the years, or by many miles, and this confirmation lends credence to the results. It is only proper to point out that although the results obtained by micropuncture may be very helpful, the methods are so difficult that they present many potential pitfalls.

We have also found (fig. 1) the proximal tubular fluid to be isosmotic, regardless of whether the final urine was hyper- or hypo-osmotic. In hydropenic rats elaborating markedly concentrated urine, the fluid in the early distal convolution is consistently hypo-osmotic, and in the second half of the distal convolution, isosmotic, but never hyperosmotic (fig. 2). Clearly, the hyperosmotic phase of urine concentration is established in the collecting ducts.

The hypotonicity of the fluid entering the early distal convolution can be explained on

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*In these and the subsequent studies, the proximal tubule is considered to extend from the glomerulus to the thin descending segment of the loop of Henle; the distal convolution, from the macula densa—where the ascending limb of the loop touches its own glomerulus—to its junction with one or more other distal convolutions to form a collecting duct.

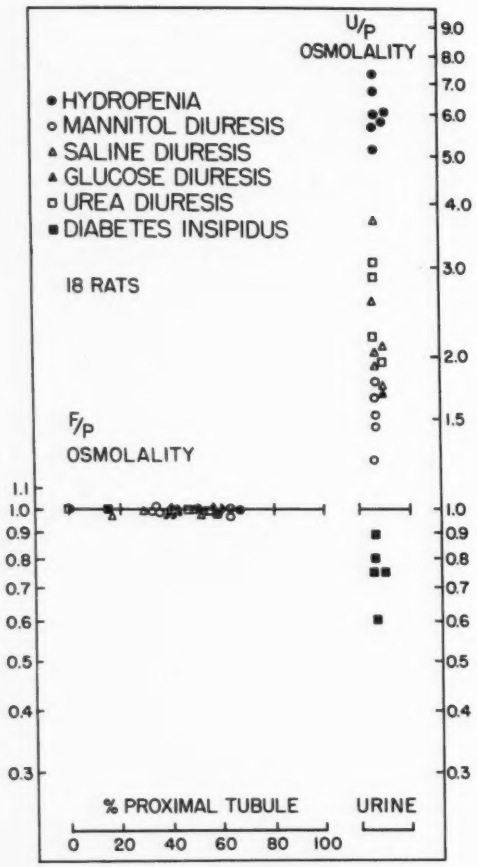

Figure 1

Osmolality ratios of proximal tubular fluid and urine in rats. (Republished by permission of the American Journal of Physiology.[5])

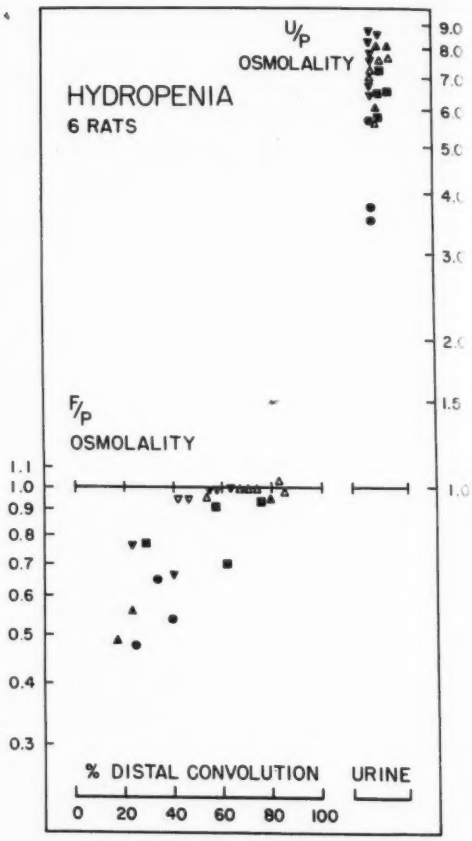

Figure 2

Osmolality ratios of fluid from the distal convolution and of urine during hydropenia. Different symbols refer to different rats. (Republished by permission of the American Journal of Physiology.[5])

the basis of hyperosmotic reabsorption of solute in the loop of Henle, or secretion of water into the loop. If it is due to reabsorption of solute in excess of water, the only solute present in sufficient quantity to explain this degree of hypotonicity is sodium chloride. Accordingly, in an attempt to differentiate between these 2 possible mechanisms, osmotic diuresis was induced with the nonreabsorbable solute, mannitol, and with sodium chloride (fig. 3).

When a 25 per cent solution of mannitol was infused intravenously, the rate of urine flow increased up to 80 times that of the hydropenic state, and the urine/plasma osmolality ratios were correspondingly low. Again, early distal fluid was hypo-osmotic, but the osmolality ratio was not less than 0.6, and late distal samples were isosmotic or nearly so. Similar results were obtained during glucose diuresis.

When sodium chloride, given as a 5 or 7 per cent solution, was the loading solute, comparable urine flows were achieved, but the degree of hypotonicity of the fluid in the early distal convolution increased. Here, a large percentage of the samples had fluid

plasma osmolality ratios less than 0.6; the minimum fluid/plasma ratio was 0.3. As before, fluid from the late distal convolution was isosmotic. The increase in the degree of hypotonicity with sodium chloride is impressive and constitutes, we believe, strong evidence that the hypotonicity of the fluid in the early distal convolution is due to hyperosmotic reabsorption of sodium chloride in the loop of Henle.

All of the data so far can be explained by the conventional theories[6] for the reabsorption of sodium and water, i.e., the proximal "obligatory" reabsorption and the distal "facultative" reabsorption of sodium and water, with the hyperosmotic phase probably resulting from active transport of water by the cells of the collecting ducts. This interpretation, however, neglects the countercurrent hypothesis of Kuhn and collaborators, which was first proposed in 1942,[7] but which had few proponents until recently. This new and revolutionary theory, treated in detail in 1951 by Hargitay and Kuhn,[8] proposes that the hairpin-like loop of Henle functions as a countercurrent multiplier system in which a small concentration gradient, at any given level in the loop, is multiplied as the tip of the loop is approached. This system was believed to result in an increasing osmotic pressure in the medulla of the kidney. The theory further proposes that the concentration of the urine results from the passive withdrawal of water from the collecting ducts as they traverse this area. Their original experimental evidence, gained in conjunction with Wirz, was a cryoscopic study of rat kidney slices.[9] They found that, at any given level, the osmotic pressure of the fluid was identical in the lumen of all the tubules. There was also an increasing osmotic gradient from the cortex, which was isosmotic with arterial plasma, to the tip of the papilla. Although the recent analyses of distal tubular fluid[4, 5] do not invalidate the concept of an increasing osmotic gradient from cortex to papilla in at least some of the medullary structures, they do demonstrate that all tubular fluid at a given level in the kidney does not have exactly the same osmolality. They also suggest to us that postmortem diffusion probably accounted for this aspect of the results of Wirz, Hargitay and Kuhn.[9] Since some doubt remained that the osmolality in all of the medullary structures exceeds that of the cortex, additional direct sampling and measurement of osmolality seemed to be required. Wirz[10] accomplished this for the medullary blood vessels

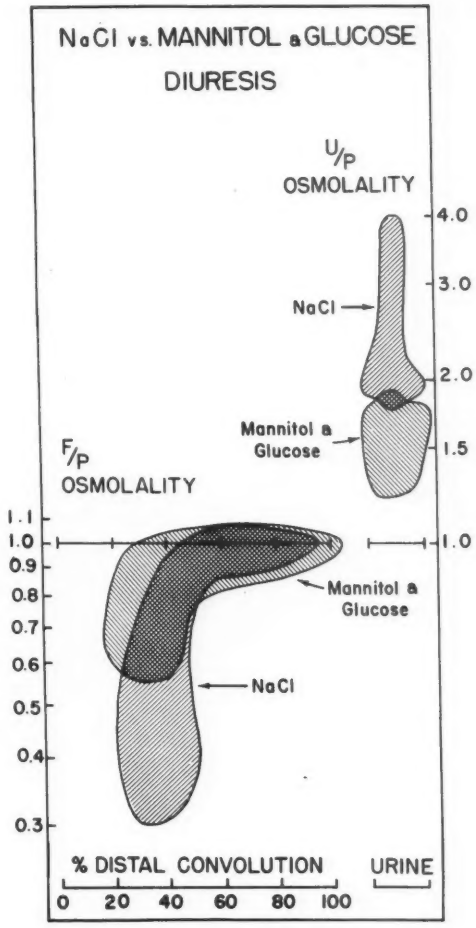

Figure 3

Comparison of the osmolality ratios of fluid from the distal convolution and of urine during hypertonic sodium chloride diuresis with the ratios during hypertonic mannitol and glucose diuresis. (Republished by permission of the American Journal of Physiology.[5])

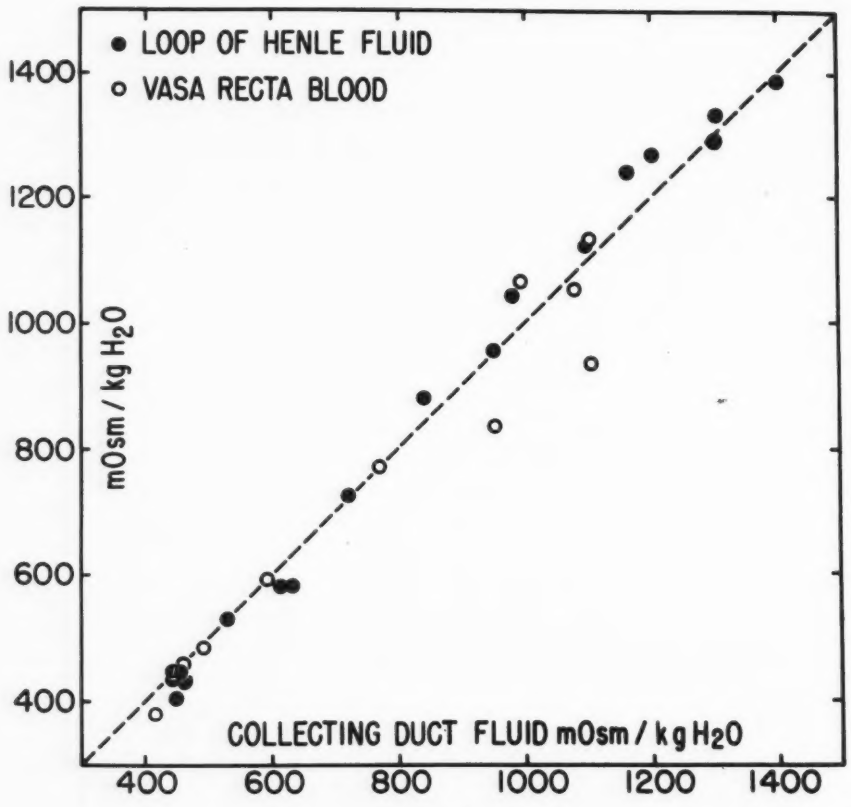

Figure 4

Relation between the osmolality of collecting duct fluid, fluid from the loops of Henle, and vasa recta blood in various desert rodents. (Republished by permission of the American Journal of Physiology.[5])

by demonstrating that blood from the vasa recta at the tip of the hamster papilla had the same osmolality as that of the final urine. More recently, we accomplished the same for fluid from the loop of Henle. Under conditions of proper illumination, it is possible to identify loops of Henle in the living hamster papilla and to sample their contents. Fluid from the bends of the loops, or very close thereto, had essentially the same osmolality as that from a collecting duct at the same level (fig. 4). These results demonstrate that the urine is first concentrated and then diluted before its final concentration, as required by the countercurrent hypothesis. We were also able to confirm Wirz's observation that the osmolality of blood from the vasa recta is the same as that of fluid from a collecting duct at the same level.

These observations indicate to us that a countercurrent mechanism is operative in the nephrons of the kidney, as well as in the vasa recta. The details of the mechanism remain to be established, and one can propose numerous variations of the countercurrent hypothesis that will explain the facts that are now available. We agree that the active transport on which the system depends is performed by the nephron, and that the loop of Henle functions as a countercurrent multiplier system. The exchanges in the vasa recta, as in other blood vessels, are probably passive and they are be-

lved to function as countercurrent diffusion exchangers, making the concentrating mechanism far more efficient. According to this view, the loop of Henle functions as a source of sodium ions for the medullary interstitium, and as a result of its hairpin-like shape and the nature of its permeability, an osmotic gradient is established in it as well as in the other medullary structures. Another general feature is that all exchanges are believed to take place between the lumina of the various structures and the adjacent interstitium, and not directly between tubular lumina. Fortunately, with such a mechanism, one is able to explain all movement of water as being secondary to previous movement of solute. Our present working hypothesis,[5] which is based on the assumption that there is no active transport of water, follows:

"Sodium, by an unknown active mechanism, and chloride, as a result of the electrochemical gradient established, are believed to be transported out of the relatively water impermeable ascending limb of the loop of Henle into the interstitium of the medulla until a gradient of perhaps 200 mOsm. per Kg. of water has been established between the fluid of the ascending limb and interstitium. This single effect is multiplied as the fluid in the thin descending limb comes into osmotic equilibrium with the interstitial fluid by the diffusion of water out of, and probably the diffusion of some sodium chloride into, the descending limb, thus raising the osmolality of the fluid presented to the ascending limb. In this fashion, an increasing osmotic gradient is established in the direction of the tip of the papilla, and yet at no level is there a large osmotic difference between luminal and interstitial fluid. In contrast, the epithelium of the collecting ducts in the presence of antidiuretic hormone is believed to be water permeable and functionally sodium impermeable (net transport is probably small although there may be diffusion into, and active transport out of, the collecting ducts). This results in diffusion of water out of the collecting ducts into the hyperosmotic medullary interstitium until the fluid remaining in the collecting ducts becomes correspondingly concentrated. It is apparent that in order for the urine to be significantly concentrated, the flow through the loops of Henle must considerably exceed the flow through the collecting ducts.[8] This is accomplished under the influence of ADH by diffusion of water out of the distal convolution into the interstitium of the cortex, reducing the volume and increasing the osmolality to the isosmotic level of the fluid presented to the collecting ducts. As suggested by Schmidt-Nielsen,[11] urea is probably also involved in some unknown fashion. It appears most likely to us that urea diffuses into the descending limb, contributing to the osmotic gradient established in the loop, and diffuses out of and/or is actively transported out of the ascending limb. Unlike sodium, depending on the circumstances, there may be net diffusion of urea from the interstitium into the collecting ducts (exaltation), or vice versa, as water is extracted from them.

"Recent work by Hilger, Klümper and Ullrich[12] indicates that active reabsorption of sodium by the epithelium of the collecting duct also occurs. This reabsorption may play an important role in maintaining a high concentration of sodium in the interstitium of the medulla by preventing loss of sodium in the urine. In the presence of ADH, reabsorption of sodium or other solute by the cells of the collecting duct would lead to the isosmotic reabsorption of water at the level at which the reabsorption of the solute occurred, and a reduction in the volume of fluid presented to any more distal portion of the collecting ducts. In the absence of ADH, reabsorption of solute would lead to further dilution of the collecting duct fluid; this view is consistent with the observations of Wirz[4] and ourselves (unpublished observations) on the osmolality of fluid from the end of the distal convolution and of urine.

"The vasa recta also participate in this mechanism, as first shown by Wirz[10] and now confirmed by us, and apparently function as countercurrent diffusion exchangers as shown in figure 5. (See Scholander[13] for a discussion of this general biologic principle.) They make

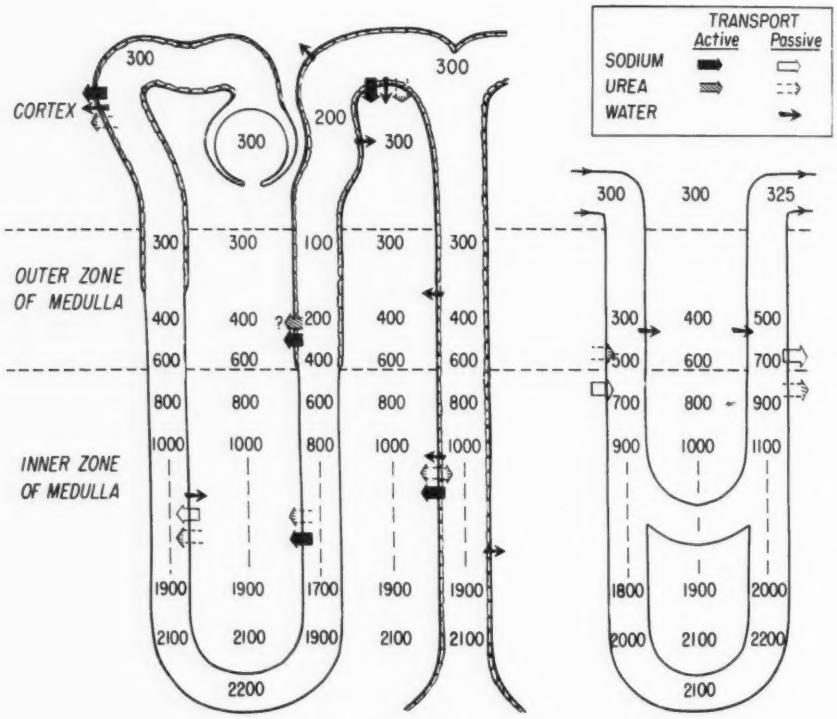

Figure 5

Diagram depicting the countercurrent mechanism as it is believed to operate in a nephron with a long loop and in the vasa recta. The numbers represent hypothetic osmolality values. No quantitative significance is to be attached to the number of arrows and only net movements are indicated. As is the case with the vascular loops, all loops of Henle do not reach the tip of the papilla, and hence the fluid in them does not become as concentrated as that of the final urine, but only as concentrated as the medullary interstitial fluid at the same level. The active sodium transport by the epithelium of the collecting duct is based on the work of Hilger, Klümper and Ullrich.[12] Kuhn[17] has recently considered the theoretic aspects of the activation of the counter-current multiplier by active sodium transport. (Republished by permission of the American Journal of Physiology.[5])

the entire mechanism far more effective, resulting in a higher osmotic gradient, by tending to trap sodium, urea and other diffusible solutes in the medulla. This aspect of the mechanism has recently been clearly discussed by Berliner and his co-workers,[14] who have emphasized the importance of a low effective medullary blood flow in establishing a high osmotic gradient. We would point out, however, that the osmotic equilibration of the blood in the vasa recta with medullary interstitial fluid in all likelihood is not due only to the diffusion of solute into their descending, and out of their ascending limbs, but also results in large part from the diffusion of water in the opposite direction. This short circuiting of water across the tops of the vascular loops may be, at least in part, responsible for the seemingly rich content of red cells in the vasa recta at the tip of the papilla. The efficiency of the countercurrent exchange in the vasa recta is critical, for they probably remove not only the blood entering the medulla, but also the water that diffuses

from the thin descending limbs of the loops of Henle and the collecting ducts. This water, with solutes isosmotic for the particular level of the medulla, presumably moves into the vasa recta because of the gradient of its chemical potential established by the colloid osmotic pressure of the plasma proteins, since the hydrostatic pressures in the capillaries and interstitium are the same.[15] The more nearly the osmotic pressure of the blood leaving the medulla approaches that of the blood entering it, the less solute will be lost from the medulla, and hence the higher the osmotic gradient established."

As noted above, many hypotheses are consistent with the presently established facts. For example, the hypothesis of Berliner et al.,[14] in which both the descending and ascending limbs of the loop of Henle were postulated to be relatively water-impermeable and actively to transport sodium from lumen to interstitium, is compatible with present information if the thin descending limb is assumed to be water-permeable in the presence of antidiuretic hormone. In such a system, the volume of fluid reaching the tip of the loop would be somewhat smaller than in the hypothesis detailed above, because transport of sodium out of the thin descending limb would lead to further extraction of water.

Ullrich[16] has proposed that the active transport of sodium in the medulla is limited to the epithelium of the thick ascending limb and collecting ducts, and that exchanges in the thin limbs are passive. He visualizes that the osmolality of the loop and of the interstitial fluids is the same throughout the inner medulla, and that osmotic equilibration with collecting duct fluid lags behind so that the fluids in the loop and in the collecting duct have the *same* osmolality only at the tip of the papilla. We believe this hypothesis is unlikely, since we have recently been able to demonstrate in a few loops in the papilla of the desert rodent, *Psammomys obesus*,* an *increase* in osmolality in the direction of their tips.

Micropuncture data on the diluting kidney are few and difficult to obtain, but proximal fluid is certainly isosmotic and early distal fluid, hypo-osmotic. In rats in which the final urine was quite dilute, Wirz[4] found that the fluid remained dilute throughout the distal convolution, and perhaps was diluted a bit further in the collecting ducts. In a small series of observations on animals elaborating urine which was only slightly hypo-osmotic, we found that the fluid was isosmotic by the end of the distal convolution and that the final dilution occurred in the collecting ducts, presumably the result of the active reabsorption of sodium chloride by a water-impermeable membrane. Additional data are obviously needed before one can be confident of the course of events in this situation.

Antidiuretic Hormone

The micropuncture findings are consistent with the hypothesis that the epithelium of the collecting ducts, and perhaps the distal convolution, is rendered freely permeable to water by the presence of antidiuretic hormone,[4, 14] but we agree with Wirz[4] that antidiuretic hormone may have an additional effect on the concentrating mechanism. If a change in the permeability to water of the epithelium of the collecting duct and of the distal convolution were its only effect, the interstitium of the medulla could be even more hyperosmotic during water diuresis than during hydropenia, for less water is probably extracted from the collecting ducts during water diuresis than during hydropenia. Among other possible mechanisms whereby antidiuretic hormone could facilitate the operation of the countercurrent system are 1 or more of the following actions: (1) increased transport of sodium by the thin, ascending limb; (2) increased permeability to water of the thin, descending limb; or (3) decreased medullary blood flow.

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The First Micropuncture Studies of the Kidney

In the spring of 1921 I had the privilege of seeing a demonstration of Professor Robert Chambers of New York in which, by means of micropipettes, he injected various substances into the cell bodies of amoebae. It excited me to believe that the frog's kidney, already made accessible to direct vision, might become accessible also to instrumental manipulation. Dr. J. T. Wearn, learning of this thought, crystallized it by suggesting that it might be possible to draw fluid from the intracapsular space within the malpighian body and thus to compare its chemical composition with that of the blood plasma from which it is derived. If such a project could be successfully and comprehensively accomplished, we should gain knowledge of the most direct character by which to estimate the nature of the processes concerned in the separation from the blood within the glomerulus of the fluid which is eventually to become urine: and a far more satisfactory basis for study of function of the tubules would be laid than existed previously because, until we are definitely certain concerning the amount and composition of the fluid which constitutes the head water of the stream flowing down the tubule, we can scarcely hope to decide with certainty concerning the changes to which this fluid is subjected during its passage through the tubule.—A. N. Richards. *Methods and Results of Direct Investigations of the Function of the Kidney.* The Beaumont Foundation Lectures, series 8. Baltimore, Williams and Wilkins, 1929, p. 15.

Function of the Collecting Ducts

By KARL J. ULLRICH, M.D.

Urine was sampled from microcatheters situated at different levels of the collecting ducts of golden hamsters. Appropriate analyses provided data concerning the function of the collecting ducts with respect to concentrating and acidifying the urine. The conclusion is reached that the collecting ducts serve 2 functions: a *passive* one, i.e., the back-diffusion of water and urea and an *active* one, i.e., the exchange of sodium for hydrogen ions and the formation of ammonia.

HEIDENHAIN[1, 1a] in 1874 injected indigo carmine into intact animals and found a strong concentration of color in the collecting ducts. His explanation was that there is relatively more urine in the collecting ducts and that some precipitated dye is collected by coagulation in a manner analogous to sand being swept along by a stream. He attributed no special function to the cells of the collecting ducts because of their clear appearance. This interpretation was accepted for 70 years.

In 1949 Vimtrup,[2] in the laboratory of Bodil and Knut Schmidt-Nielsen, repeated experiments with dyes on *Heteromyidae* whose collecting ducts are very long and a good subject for experimental study. He concluded that the urine becomes concentrated as it flows through the collecting ducts. In 1951 Hargitay and Kuhn[3] published their countercurrent hypothesis, showing by experiments performed in conjunction with Wirz,[4] that the urine was concentrated in the collecting ducts.

To obtain more information about the function of collecting ducts, Hilger, Klümper, Eigler, Pehling and Ullrich[5-8] catheterized the collecting ducts of golden hamsters, whose papillae are easily accessible from the renal pelvis. The experimental procedure was as follows: 2 polyethylene microcatheters were passed from the renal pelvis into 2 collecting ducts, the tips reaching different levels. Both samples of urine which were obtained in this way were analyzed with respect to the depressions of the freezing point and the concentrations of inulin, sodium, and potassium.

The results are plotted in figure 1. In 33 cases, the concentration of inulin in urine from the superficially placed catheter averaged 1.5 times that from the deeper one. In a second series of animals with higher urinary osmotic pressure, the mean increase was threefold. If it is assumed that the increase in inulin concentration is due to the reabsorption of water, the results of both series indicate that between one-third and two-thirds of the water flowing into the portions of the collecting ducts under consideration is reabsorbed.

If water alone is reabsorbed from the fluid of the collecting ducts, the increase in osmotic pressure ought to parallel the increase in the concentration of inulin. But (fig. 2), such parallelism was not observed: the real osmotic pressures increase with much less of a slope. From this discrepancy one can conclude that some solutes must be reabsorbed too, thereby making the osmotic pressure of the reabsorbed solution equal to about two-thirds of that of the solution flowing into the corresponding segment of the collecting ducts.

In other experiments, Klümper, Ullrich, and Hilger[6] measured the concentrations of inulin and urea in samples obtained from microcatheterization of collecting ducts. In all cases the increase in the concentration of inulin exceeded that of urea. The ratios of the rate of increase in the concentration of urea to the rate of increase in the concentration of inulin varied from 0.37 to 0.97, with an average of 0.65. These data indicate that the con-

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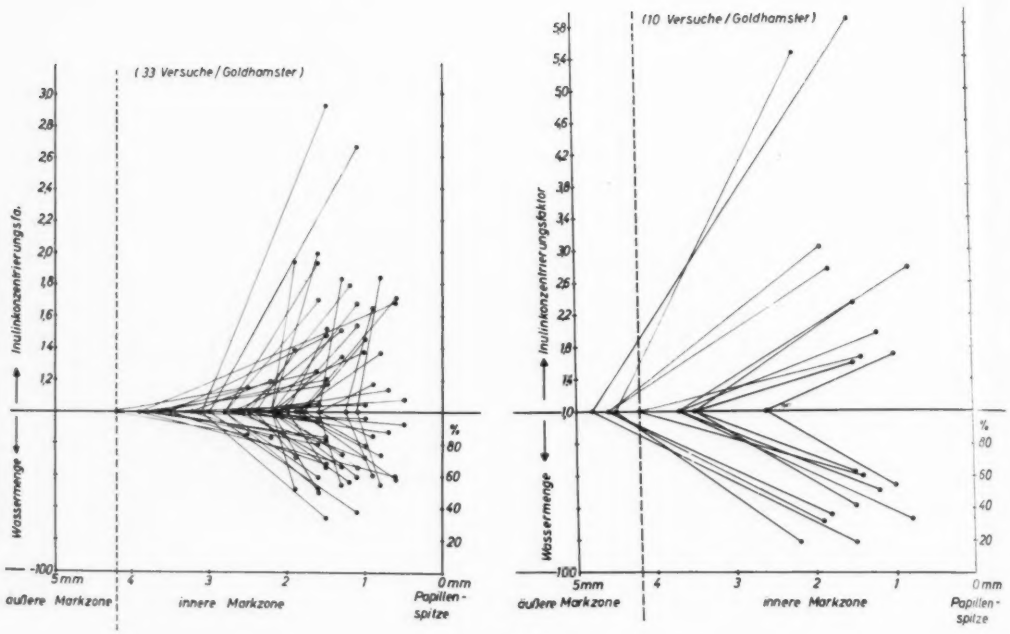


Figure 1

Relative changes in the concentrations of inulin in the urine within the collecting duct, and the corresponding reabsorption of water. The data illustrated on the right side of the figure are from animals excreting a more concentrated urine than those on the left. On the abscissa are plotted the distances of sampling points from the tip of the papilla. The straight lines represent the values of the simultaneously gained samples. Data taken from Hilger, Klümper and Ullrich.[5]

centration of urea in the reabsorbed solution is approximately half the concentration of the fluid flowing into the particular segment of the collecting duct from which samples were obtained.

What changes occur in the electrolytes during the passage of urine through the collecting ducts? On the average, the increases in the concentrations of potassium parallel the increases in the concentrations of inulin.[5] This supports the view that potassium is concentrated only by reabsorption of water in the collecting ducts of the inner medullary region.

The concentrations of sodium in the urine, however, fall as the urine passes through the collecting ducts.[5] As may be seen in figure 3, there is a steep slope, with high values for concentration in the outer medullary region. This fact indicates that a great deal of water is reabsorbed from the collecting ducts of the cortex and the outer medulla. The sodium concentrations are very low at the papillary tip. Thus, sodium must be reabsorbed from the collecting ducts. We may then conclude that the collecting ducts can regulate the excretion of sodium by the kidney.

But how can this drop in the concentration of sodium be reconciled with the coincidental rise in the osmotic pressure of urine in the same region? Simultaneous measurements of the concentrations of inulin and ammonium by Ullrich, Hilger, and Klümper[8] indicate (fig. 4) that ammonium increases much more than inulin. Therefore, we may conclude that ammonium ions are secreted into the collecting ducts. In connection with this observation, it is interesting to note that Richterich-van Baerle, Goldstein, and Dearborn[9] have le-

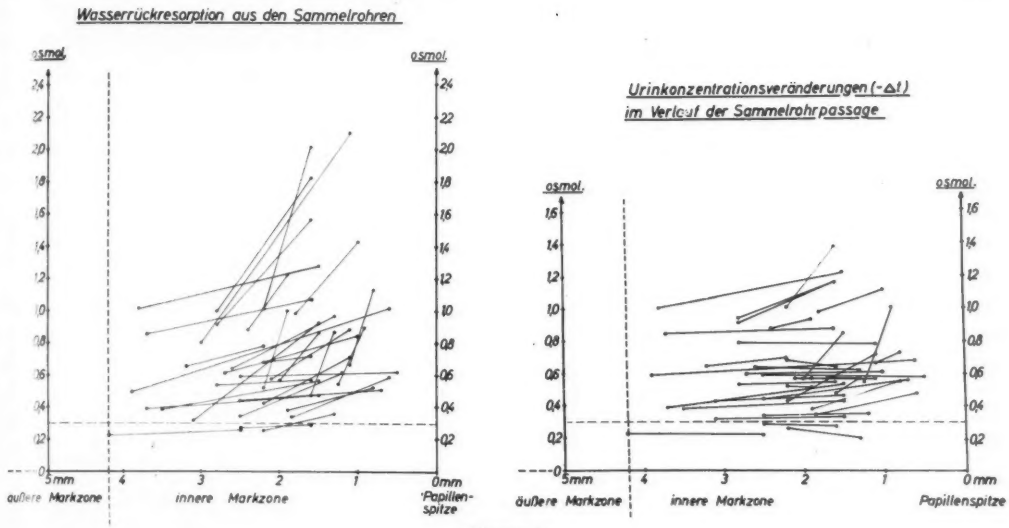
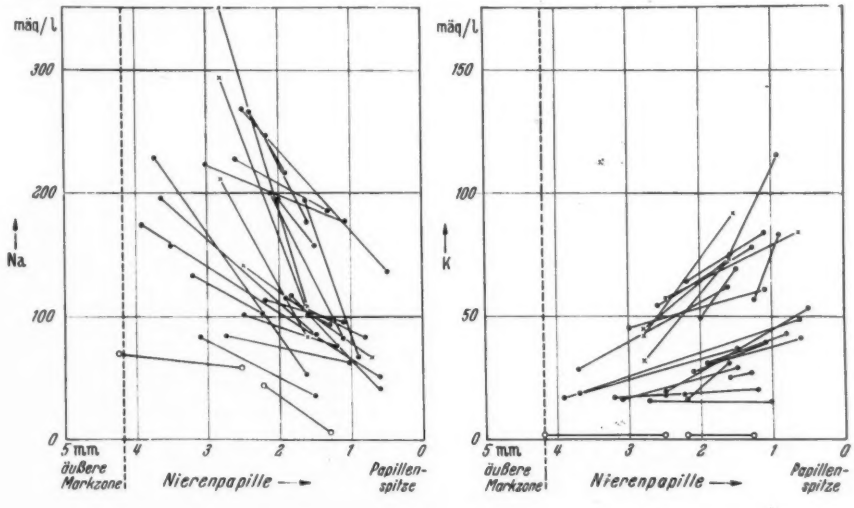


Figure 2

Changes in the osmotic pressure of urine along the course of the collecting duct. The data used for the left side of the figure were calculated from the freezing-point depression of urine obtained from the deeper level and the respective concentrations of inulin in urine from both levels. This hypothetic figure would be expected if only water were reabsorbed. The observed increases in osmotic pressure (right) are less. Data taken from Hilger, Klümper and Ullrich.[5]



a b

Figure 3

a. *Changes in the concentrations of sodium during the passage of urine through the collecting duct (26 experiments on golden hamsters). On the abscissa is plotted the distance of the sampling point from the tip of the papilla.* b. *Changes in the concentrations of potassium during the passage of urine through the collecting duct (23 experiments). (Republished by permission of Pflüger's Archiv für die gesamte Physiologie des Menschen und der Tiere.*[5]*)*

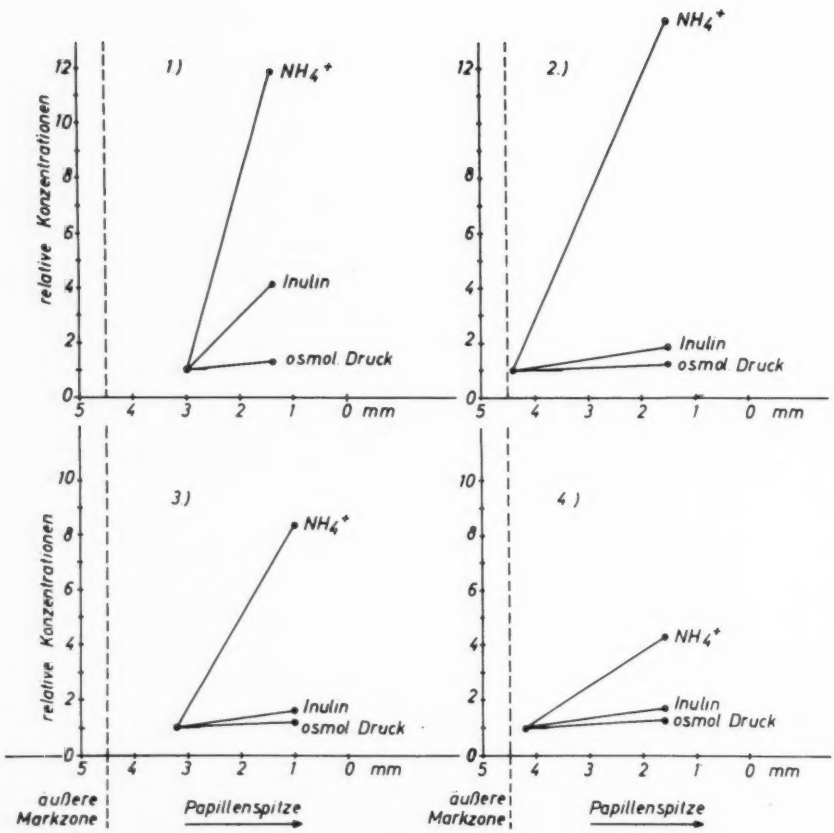


Figure 4

Changes in the concentration of ammonia in urine during the passage through the collecting duct compared with the changes in the concentration of inulin and in the osmotic pressure in the same samples (4 representative experiments). Data taken from Hilger, Klümper and Ullrich.[5]

scribed a high degree of activity of glutaminase I in the inner zone of the renal medulla of dogs and rabbits. Moreover, as may be seen in figure 5, acidification of urine within the collecting ducts was demonstrated by a decrease of pH.[7] These observations were recently confirmed by Gottschalk[10] and Giebisch,[11] who have also shown that acidification can take place in the proximal and distal convolutions. The present data indicate that there is an exchange of sodium ions for hydrogen ions and a secretion of ammonia in the collecting ducts.

The findings by the stop-flow method[12-15] are in accord with our results as far as the reabsorption of sodium and the secretion of hydrogen ions and ammonia are concerned. However, a discrepancy exists with respect to the site of secretion of potassium. According to the stop-flow method, potassium is secreted at the same site as hydrogen ions and ammonia. However, our results indicate that potassium is not secreted in the collecting ducts of the inner medullary region, but that secretion of potassium may occur at a site higher up in the collecting ducts.

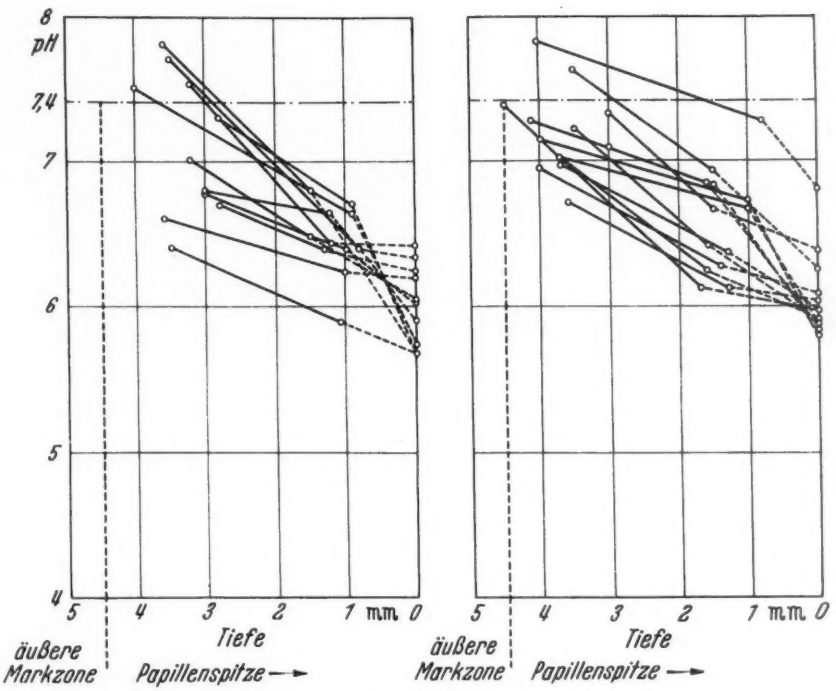


Figure 5

The pH of urine drawn simultaneously from the collecting duct at different distances from the tip of the papilla. The graph on the left represents values of samples withdrawn without special precautions. The values for the graph on the right were measured at a carbon dioxide tension of 40 mm. Hg. The values at abscissa point 0 are values from the other (unexposed) kidney. (Republished by permission of Ergebnisse der Physiologie, Biologischen Chemie und Experimentellen Pharmakologie.[7a])

To summarize, the collecting ducts seem to have 2 functions: (1) a *passive* one, i.e., the back-diffusion of water and urea, and (2) an *active* one, i.e., the exchange of sodium ions for hydrogen ions and the formation of ammonia. Both functions, passive and active, seem to operate independently. It is particularly surprising that the cells of the collecting ducts, which are exposed to enormous changes in the concentrations of electrolytes and urea, and are threatened by an inadequate supply of oxygen and essential nutrients as a consequence of countercurrent diffusion, are entrusted with such essential functions as the exchange of sodium and hydrogen and the secretion of ammonia.[7a]

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The Animal that Fits

In 1926, Marshall, browsing through comparative anatomy, discovered that a number of fishes had been described which possessed purely tubular kidneys. This fact, of course, immediately evoked Marshall's interest in fish urine. Unfortunately, aglomerular fishes were rather rare, but we happened to have one species, the goosefish, at Salisbury Cove . . .

Work on the aglomerular fishes had been undertaken independently by J. G. Edwards in the Naples' laboratory, and within a short time it was clear that this purely tubular kidney, which does not even possess a significant arterial blood supply, but is perfused entirely by venous blood from the renal portal vein and at a pressure which is probably below the osmotic pressure of the plasma proteins, can excrete all the ordinary urinary constituents . . .

So, by 1930, the question of tubular excretion was answered in the affirmative. But, as Richards said in the discussion when Marshall read a paper at Woods Hole on the aglomerular fish, "At last he has found an animal that fits in with his theory."—H. W. Smith. *Lectures on the Kidney.* Lawrence, Kansas, University of Kansas Press, 1943, p. 73.

Discussion

Dr. Berliner: I should like to comment upon the last item covered by Dr. Gottschalk, that is the question of whether there is any necessity for assuming that antidiuretic hormone has any other effect than on the loss of water in the distal tubules and in the collecting ducts. I agree that if we considered only the transport of sodium by the loops, and the concentration of the urine, we would predict, on the assumption that the interstitial space is less diluted by water from the collecting ducts, that the interstitial fluid would be more hypertonic during water diuresis than during antidiuresis. But during antidiuresis, about half of the osmotic pressure is due to urea, which is not present during water diuresis. Since we are left only with sodium and chloride, the interstitial fluid would have a considerably lower osmotic pressure during water diuresis than during antidiuresis—at least as far as *total* osmotic pressure is concerned.

However, it is entirely possible the animal undergoing water diuresis loses more water from the collecting ducts than does the hydropenic animal putting out a very small volume of water. In the animal undergoing water diuresis, the urine entering the collecting ducts is already dilute; if the urine then loses some water in the collecting ducts, it will be difficult to determine how much water has been lost, since we don't know how much entered the collecting system. We are certainly not justified in considering the collecting ducts totally impermeable to water during water diuresis.

On the other hand, in the animal putting out a very small volume of highly concentrated urine, we can make a rough estimate of how much water was lost from that urine in the collecting system. Even allowing, in accord with the studies of Dr. Ullrich, for the loss of a certain amount of solute from the collecting ducts, the amount of water which is removed turns out to be very small. It is entirely possible, therefore, that the water removed during water diuresis is actually greater than during antidiuresis, and, if this is so, the fluid in the interstitial space will have a lower sodium concentration than during oliguria.

I certainly agree with Dr. Gottschalk that the blood flow through the medulla may increase during hydration. Such an increase would tend to reduce the osmolality of the interstitial fluid during water diuresis.

Dr. Gottschalk: I would agree completely with Dr. Berliner's interpretation that, in the absence of antidiuretic hormone, the passage of urea down collecting ducts which are impermeable to urea would have a major effect on the osmotic pressure of the interstitium. I also think that it is possible to obtain data which will allow one to quantify, reasonably well, the magnitude of the water movement under the different conditions which he has considered. The main difficulty which we have encountered in doing such experiments has been in the establishment of a steady-state water diuresis in animals which have been anesthetized and exposed to extensive surgery.

Dr. William Lassiter, who has been working with us for the past couple of years, now has very good evidence to support the concepts and the data of Dr. Ullrich on the removal of solute from the collecting ducts; for, he also has found, in a comparison of the final urine with that of fluid from the end of the distal convolution, that there is a much greater rise in the inulin concentration than in either the total osmotic concentration of the urine or in the urea concentration.

Dr. Smith: I would like to ask either Dr. Gottschalk or Dr. Ullrich if they are yet prepared to make an estimate of the relative quantity of tubular urine which is reabsorbed in the proximal loop of the distal convoluted tubule and in the collecting ducts during antidiuresis.

Dr. Gottschalk: Dr. Lassiter has accumu-

lated a fair amount of data on the inulin concentration of proximal tubular fluid. This is as determined with C^{14}-labeled inulin. It is not surprising that his data agree very closely with those of Walker, Bott, Oliver, and MacDowell, and indicate that approximately 20 per cent of the filtered water still remains at the end of the proximal convoluted tubule.

We have a few calculations on the probable osmolality of the fluid removed from the collecting ducts during antidiuresis. These calculations are certainly highly speculative, as they are not based on many facts, but it would appear that the mean osmolality of the fluid removed from the collecting ducts is about 200 mOsm./Kg. H_2O.

I would like to ask Dr. Ullrich a question about 1 of his slides, which showed a very high concentration of sodium in the upper regions of the collecting duct. Wasn't the concentration 300 mEq./L.?

Dr. Ullrich: Yes, the concentration was up to 300.

Dr. Gottschalk: Could you tell us more about this?

Dr. Ullrich: My impression is that in the outer medulla, there must be more reabsorption of water than of solutes, or there must be a back-diffusion into the collecting duct of the sodium which was pumped out by the thick ascending limb. These are the 2 possibilities, but I cannot distinguish between them.

Dr. Hilton: I may have missed it, Dr. Gottschalk, but is there any difference in the osmotic pressure of urine in the papilla during low and high urine flows?

Dr. Gottschalk: Oh, yes. As judged by the osmolality of loop fluid and vasa recta blood, there is a very wide range of osmotic pressure. For instance, I showed a slide in which the fluid had an osmolality varying from approximately 400 to 1,400 mOsm./Kg. H_2O. The dilute samples were taken during profuse glucose diuresis in hamsters; during antidiuresis the osmolality was obviously much higher.

Dr. Hilton: According to the theory that you propose, during antidiuresis, the osmotic pressure in the interstitium should be somewhat lower than during diuresis. Is that correct or am I wrong?

Dr. Gottschalk: No, that is wrong. The osmotic pressure in the medullary interstitium is believed to be higher during antidiuresis than during diuresis. In the presence of antidiuretic hormone, the osmotic pressure is believed to be approximately the same in the loop of the loop of Henle and in the adjacent interstitium—at all levels of osmotic pressure. So long as ADH is present, the osmolality of the collecting duct fluid would also be the same unless the rate of urine flow were very high.

Dr. Hilton: Well, that is my question. Is it? Do the osmotic pressures follow the theory?

Dr. Gottschalk: The measurements that have been obtained so far are consistent with this, yes.

Dr. Hilton: Are they inconsistent with the secretion of water?

Dr. Gottschalk: Are they inconsistent with the secretion of water? Our over-all concept of the concentrating mechanism is based on the hypothesis that there is no active transport of water.

Dr. Hilton: I know, but have you ruled it out?

Dr. Gottschalk: Our data cannot rule it out.

Dr. Gilman: I would like to turn to a subject which has been touched upon by Dr. Ullrich. Dr. Ullrich mentioned that he was somewhat surprised, since the PCO_2 is apt to be so low, at the large metabolic activity of the collecting duct. The low PCO_2 relates, of course, to the countercurrent nature of the vasa recta; just as oxygen tensions tend to be low, PCO_2 would tend to be high.

If the last exposure of the urine is to an area of presumably high PCO_2, unrelated to the PCO_2 of arterial blood, should we pay any attention whatsoever to the vast literature that relates urinary PCO_2 to arterial PCO_2?

Dr. Ullrich: As an explanation for high CO_2 pressures in the urine, Pitts has suggested: (1) that in the process of bicarbonate reabsorption and urinary acidification there is an exchange of Na^+ ions against H^+ ions;

and (2) that in the reaction, $H^+ + HCO_3^- = H_2CO_3 = H_2O + CO_2$, the last step may be delayed. This view has been challenged by Herodsky, Miley, Kaim and Shah who based their calculations on the assumption that the CO_2 pressure in the urine is always as high as in the renal venous blood. It seems to me that if one takes into account the neat anatomic arrangement for the countercurrent exchange of gases, then a high CO_2 pressure in the urine may be attributed to Dr. Pitts' idea of delayed dehydration plus countercurrent exchange.

On the other hand, a low CO_2 pressure in the urine may be a consequence of a low supply of carbon dioxide to the medulla from metabolism and from the loops of Henle and the capillary loops. Since the urine is acidified in the collecting ducts, the chemical reaction cited above may shift to the left. The CO_2 pressure in this entire area may then drop due to countercurrent diffusion, and the CO_2 pressure in the urine may fall below that in the arterial blood. The diffusion of ammonia into the collecting duct may also reduce the CO_2 pressure.

With respect to oxygen tensions, the data of Rennie, Reeves and Pappenheimer indicate that the oxygen tension in urine may be low, varying from 6 to 60 mm. Hg, with a mean of 36 mm. Hg. These data, coupled with the circulation times of Levy and Sauceda, indicate that there is a countercurrent diffusion of O_2 as well as of CO_2.

Dr. Wilde: Concerning the source of the sodium supplied to the papillary interstitium, I think Dr. Ullrich's data in the hamster warrant special mention. These indicate, I believe, that the sodium removed from the portion of the collecting duct from which he samples is about equal to the total quantity which comes out in the final urine. Of course, he is dealing with a low urine volume and a small quantity of sodium; nonetheless, this quantity may be a very important one in that it actually determines how much sodium is finally lost to the urine in these organisms.

Dr. Ullrich: If one extrapolates these data, the concentration of sodium in the urine approaches zero; this means that the reabsorption of sodium in the collecting duct is high. Indeed, one can calculate that the concentration of sodium in the fluid, which is reabsorbed in the collecting duct, is about 400 mM/L. Almost all of the reabsorbed sodium ions are exchanged against hydrogen ions and ammonia.

To explain the increase of osmotic pressure in the countercurrent system of the inner medulla, several authors have suggested that an outward flow of NaCl occurs on the ascending side of the loops of Henle and that an inward flow of NaCl occurs on the descending side. This would imply that the thin limb of Henle changes its function at the tip of each loop; to me, this seems unlikely. From the standpoint of morphology there is no reason to believe this hypothesis.

Therefore, I wonder if the process of acidification, or other metabolic processes in the inner medullary region, could contribute osmotically active substances to this area? Such a contribution is conceivable when one realizes that an osmotically inactive substance, carbon dioxide, after hydration and exchange of hydrogen against sodium, yields 2 osmotically active particles (Na^+ and HCO_3^-). Also, other processes such as the split of glucose to lactic acid and of glutamine to ammonium glutaminate may generate osmotically active particles in this area.

Dr. Malvin: Dr. Ullrich has already stolen a little bit of my thunder in reporting that his results with potassium seemed to disagree with the data from stop-flow. My only comment at this time is that this discrepancy may be a species difference; that is, the flow experiments in our laboratories have been performed on dogs, whereas, if I remember correctly, you have used the hamster and rat.

I should also like to ask this question: Where, then, do you visualize potassium secretion to take place?

Dr. Ullrich: I don't know where the potassium secretion may take place. It may be in the collecting duct of the outer medulla or in the distal tubule.

Chairman Pitts: I would like to ask Dr.

Ullrich, along these same lines, if there was any evidence of overt potassium secretion in his animals at the time that he collected the samples; does he have data to indicate whether the potassium clearance was approaching, or especially, exceeding the inulin clearance? In other words, is there definite evidence for potassium secretion?

Dr. Ullrich: No, there is no evidence for potassium secretion. We have seen potassium values in urine up to 50 or 60 mEq./L.; if we assume the corresponding potassium values in blood to be 5, then we are left with a U:P ratio of 10. The U:P ratio of inulin was much higher.

Dr. Smith: Everything now is pretty well mixed up with everything else in the collecting ducts, but I remain completely mixed up about 1 slide which Dr. Gottschalk showed on the diabetes insipidus animal. Do I quote you correctly in saying that in diabetes insipidus the fluid pressure is isosmotic to the plasma?

Dr. Gottschalk: The few data that we have show exactly that at the end of the distal convolution.

Dr. Smith: What are you going to do about that?

Dr. Gottschalk: Well, before one can be sure of what happens here, we need more data. I think it is very important to point out, though, Dr. Smith, that our animals were putting out urine that was only slightly hypoosmotic. The findings in the distal convolution may vary considerably depending on the degree of dilution of the final urine. I have no reason to doubt our data or Dr. Wirz's data. Perhaps the divergence in the data depends on how much antidiuretic hormone was present. Perhaps our animals had a certain amount of antidiuretic hormone, and his had practically none.

Dr. Smith: It remains important to determine the quantities of all elements which are transported in the proximal loop, the distal convoluted tubule, and in collecting ducts. We have, as yet, no idea of relative amounts of transport.

Dr. Gottschalk: I would agree 100 per cent. I think it is also important to emphasize, particularly with regard to the distal convolution, that the fluid in all convolutions is not going to be the same. In an animal such as a rat, in which the loops have dipped into the medulla for varying degrees, the inulin concentration, for instance, in distal fluid would be expected to vary considerably.

Dr. Smith: Maybe that slide should never have been used then. It may refer to different populations in the rat.

Dr. Gottschalk: That is possible.

Dr. Khalil Wakim (Rochester, Minn.): I should like to ask Dr. Gottschalk how he induced diabetes insipidus in his rats and if they ingested plenty of water?

Dr. Gottschalk: I am indebted to a gentleman who may be in the audience for the gift of these 3 animals—Dr. Robert Winters. He induced diabetes insipidus by producing hypothalamic coagulation lesions. I wouldn't for 1 minute, and I am sure he would agree, wish to imply that they had complete diabetes insipidus, and that they had no secretion at all of antidiuretic hormone.

Dr. Wakim: Did they take plenty of water?

Dr. Gottschalk: Oh, yes. I can't quote their voluntary water intake, but it was very, very large.

Question: Has the Schmidt-Nielsen laboratory found any further evidence for the active secretion of urea into the urine of these animals?

Dr. Ullrich: We have not, during the time that I have been in the laboratory, done any new experiments. All the other data have been published by Dr. Bodil Schmidt-Nielsen; she has found indications that there *may* be active secretion of urea, but these are indications only.

Measurements of Electrical Potentials and Ion Fluxes on Single Renal Tubules

By GERHARD GIEBISCH, M.D.

Experimental technics are described which permit the measurements of stable electrical potential differences across the cell membrane of single renal tubule cells and across single tubules. The inside of the tubule lumen is normally found to be electrically negative to the outside, while the cell interior is found to be negative to both the peritubular fluid and the tubule lumen. Such electrical measurements, in conjunction with known concentration gradients and flux measurements of various ions across single renal tubules, permit some deductions to be made on the driving forces involved in the movement of various ion species across the luminal and peritubular cell membrane of renal tubule cells.

RENAL function studies have indicated that among biologic membranes involved in the transport of electrolytes, the renal tubules are exceedingly active in the net transfer of most ion species. However, in spite of the establishment of considerable concentration gradients across various tubular segments and the likelihood of different transfer rates of charged particles from the lumen to the peritubular fluid, factors favoring the creation of electrical potential differences, renal tubular structures have received relatively little attention as far as electrical phenomena are concerned. Also, little is known regarding the magnitude and the kinetics of bidirectional ion fluxes across renal tubules, although site and steepness of various ionic concentration gradients have been fairly well outlined by micropuncture studies on individual nephrons.

Some of these problems have recently been studied by a combination of microelectrode technics and the perfusion of single renal tubules with solutions of known composition containing various isotopes. Also, double-perfusion of an amphibian kidney with different electrolyte solutions has yielded a reasonably stable and viable preparation which permits one to study the effects of various ions on electrical potential gradients. This presentation will be concerned with observations made on single nephrons of Necturus, an amphibian in which the size of the nephron is adequate to permit perfusion of single proximal tubules and in which stable electrical potential differences can be recorded, not only across the tubular wall but also across the cell membrane of individual tubule cells.

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The author is an Established Investigator of the American Heart Association.

Part of this material has been presented at a symposium on renal aspects of electrolyte transport at Göttingen, Germany, August, 1959.

Electrical Potential Measurements on Single Renal Tubules

I should like to discuss, first, electrical phenomena which we observed on single renal tubules. The purpose of this study was twofold: first, to investigate whether an electrical potential difference exists across the renal tubular wall and to what extent the maintenance of such a potential difference is dependent on the presence of various ion species. Our second aim was to measure transmembrane potentials of single renal tubule cells, and if possible, to evaluate permeability characteristics of the luminal and peritubular cell side from ionic concentration gradients and electrical potential measurements before and after specific changes of the ionic environment had been induced.

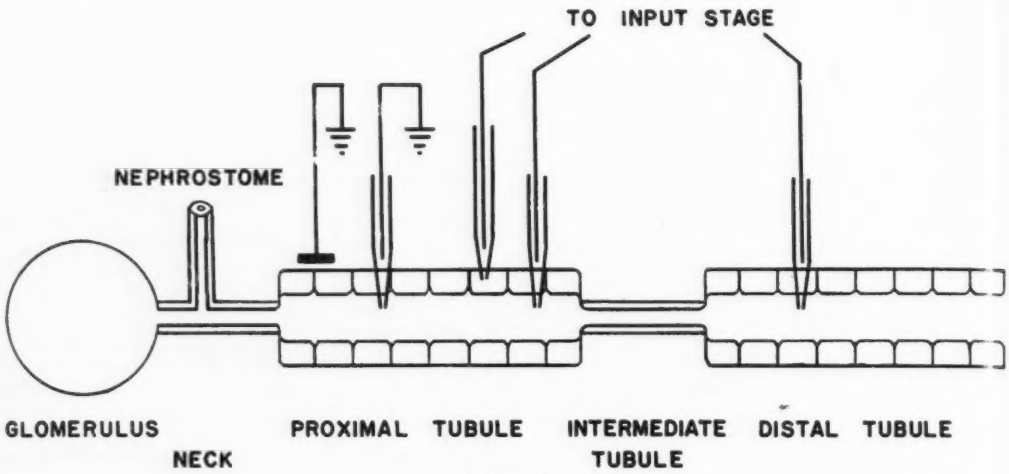

Figure 1

Single tubule of Necturus showing relation of indifferent and recording microelectrode to various tubular structures.

Figure 1 shows a schematic view of the site of the microelectrode tip in relation to renal tubular structures. When a glass microelectrode having a tip-diameter of less than 1μ. is lowered upon the kidney, a "transcellular" potential difference (i.e., a transmembrane potential of a single tubule cell) can be recorded before the microelectrode gains access to the tubule lumen and records a "transtubular" potential difference. Note the site of the indifferent electrode. During most measurements, the indifferent electrode was located on the surface of the kidney, effectively grounding the extracellular fluid. In a number of experiments, however, we have chosen to place a Ringer-agar micropipet into the tubule lumen and record differentially between tubule lumen and cell interior. This specifically permits one to test the effect of changes in the composition of tubule fluid on the potential difference between tubule lumen and cell interior across the luminal cell membrane. Attention should be drawn to the possibility that part of a transtubular potential difference is shunted through the glomerulus, the nephrostome, or through lower parts of the nephron. Fortunately, direct evidence indicates that this does not occur to any significant extent. When a column of colored mineral oil is injected into the proximal tubule and split by deposition of a small amount of Ringer's fluid, the glomerulus, the neck, the proximal end, as well as the most distal part of the tubule, are filled with oil. The transtubular potential difference under these conditions is identical with that recorded across a nonperfused tubule.[1-3]

Figure 2 summarizes data obtained in anesthetized Necturus on the frequency distribution of renal tubular transcellular potentials.[1] Stable measurements can be maintained for as long as 45 minutes. Results obtained in animals with undisturbed renal circulation are compared with those having spontaneously sluggish renal circulation or complete renal vascular stasis. Also included are measurements on animals pretreated with various amounts of radiochlormerodrin (Neohydrin). Some of these animals had renal ischemia. Calculation of the mean of 113 observations gave a mean electrical potential difference across the membrane of single renal tubule cells of −72 mV (s.d. of the mean ± 7.2 mV). Significantly lower values were obtained in animals pretreated with chlormerodrin 24 to 48 hours prior to the experiment in doses effecting a

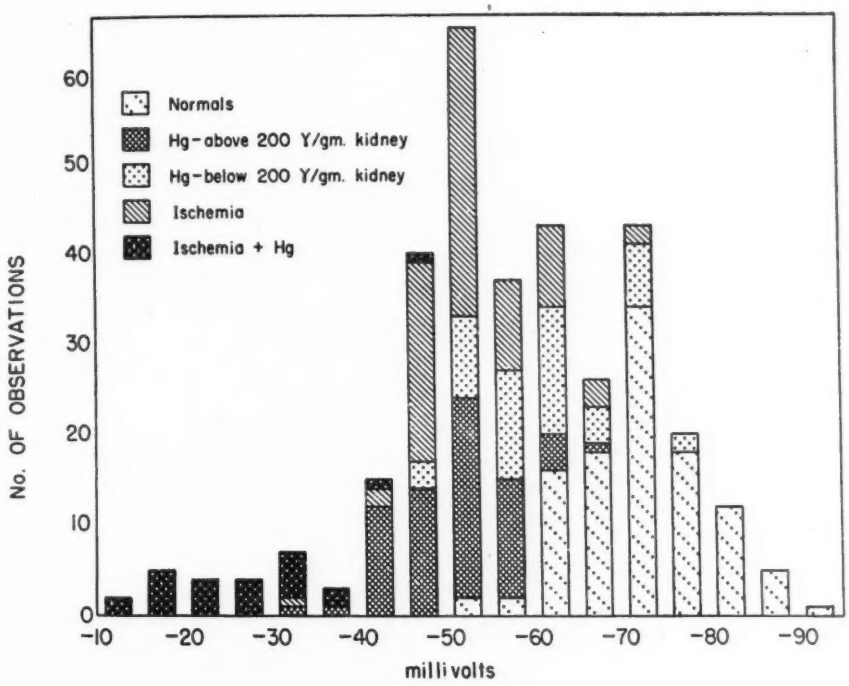

Figure 2

Summary of transmembrane potentials of single tubule cells. Values of electrical potentials are plotted, pooled in groups of 3 mV, against the number of observations. (Republished by permission of the Journal of Cellular and Comparative Physiology.[1])

mercury content of the kidney of more than 200 γ/Gm. kidney. Smaller amounts of mercury were less effective in reducing cellular transmembrane potentials. It is also apparent that impairment of the renal circulation depresses a fraction of the normal membrane potential (most potential measurements on ischemic kidneys yielded values ranging from −45 to −55 mV). Such a state of ischemia, lasting for even as long as 5 to 6 hours, does not lead to a progressive decline of renal tubular cell potentials. A phase of partial depolarization within the first 30 minutes is followed by stabilization of the potential at a lower level of about 65 to 70 per cent of the original values obtained before ischemia had begun. Additional observations on transcellular potentials of single tubule cells were obtained in the doubly perfused kidney of Necturus.[4] This preparation was found to be valuable in testing the effects of changes in the composition of the medium surrounding the tubule cells on transcellular and transtubular potential differences. We have, in essence, followed the technics as originally described by Cullis[5] and Hoeber's school,[6] in which the aorta, anterior abdominal vein and postcaval vein were cannulated and perfused with oxygenated Ringer's solution. Effluent fluid from the postcaval vein was allowed to drain freely and, in some experiments, its electrolyte composition was analyzed for sodium, chloride and potassium. Either a bicarbonate- or phosphate-Ringer was used for control experiments. From a number of function studies it appears that this preparation is stable and viable for several hours.

The first modification of the perfusion fluid which was undertaken was to change the potassium concentration in the portal and

aortal perfusion systems. The results of measurements of peritubular transcellular potential differences of proximal tubule cells may be summarized by stating that above a concentration of 5 mEq./L., there is a straight-line relationship between transmembrane potential and the logarithm of external potassium concentration. A slope of 53 mV per tenfold change in potassium concentration is observed. Below an external potassium concentration of 5 mEq./L., the transcellular potential difference increases, but deviates from the linear relationship which might be expected if the potential difference were solely due to the potassium concentration gradient across the cell membrane. We conclude from these results that the peritubular transmembrane potential difference of single proximal tubule cells is essentially like that to be expected from a potassium concentration cell. Stated differently, the fact that potassium ions depolarize renal tubule cells is indicative of considerable permeability of the peritubular, contraluminal cell side to potassium, permitting this cation to discharge the intracellular negativity when its concentration in the extracellular fluid is increased.

The fact that depolarization measured across the peritubular cell membrane occurs when the potassium concentration is increased does not permit one to decide directly to what degree the luminal side of the cell is permeable to potassium. Therefore, we have attempted to measure the effect of an increased potassium concentration on the electrical potential difference between lumen and cell interior in the following manner. A kidney of Necturus is perfused via aorta and portal system with fluid containing potassium at the normal level of 4.5 mEq./L. Next, a glomerulus is punctured and the adjoining single nephron perfused with a solution of high potassium concentration. This solution is colored by the addition of T 1824 (Evans blue) to give a sharp outline of the nephron and to indicate any excessive leakage of the perfusion fluid from puncture sites. A second micromanipulator is used to insert a fine-pointed glass capillary, filled with Ringer-agar, into the lumen of the perfused tubule, constituting the indifferent electrode. Finally, a third manipulator permits one to insert the recording microelectrode intracellularly. By this arrangement the effect of potassium ions on the electrical potential difference between tubule lumen and cell inside is measured directly. Expressed differently, it is possible to test whether or not potassium ions placed within the tubule lumen enter the cell at the luminal border and discharge its negativity. Although more data are needed, experiments performed in this way indicate that the renal tubule cell is permeable to potassium also at the luminal side, since increasing depolarization can be observed when the potassium content of the tubule lumen is raised in a stepwise fashion.

Figure 3 contains a summary of transmembrane potentials of single renal tubule cells as measured in nonperfused and in perfused kidneys of Necturus. On the left a summary is given of what has been discussed previously, demonstrating a normal mean value of −72 mV in the nonperfused kidney, and various degrees of depolarization effected by the administration of Neohydrin and the development of ischemia. On the right is a summary of our data obtained in the perfused Necturus kidney. So far, some 70 perfusions have been performed. The mean of 427 intracellular impalements gives an intracellular negativity of 64 mV. Again, the addition of Hg^{203}, as labeled Neohydrin, to the perfusion fluid reduces the cell potential. We have also performed perfusions using a modified phosphate-Ringer, in which sodium was replaced by choline, and others in which sodium chloride was replaced by sucrose. Finally, we have substituted sulfate for chloride and observed a small degree of cell depolarization. However, considerably more depolarization was seen when sucrose was the main osmotic constituent, or in particular when choline chloride was used. The depression of transcellular potentials by the latter substitution to the degree observed is noteworthy and, particularly in the case of choline chloride, in contrast to observations made on nerve and muscle.[7-9] Experiments performed by Maizels and Remington[10] on

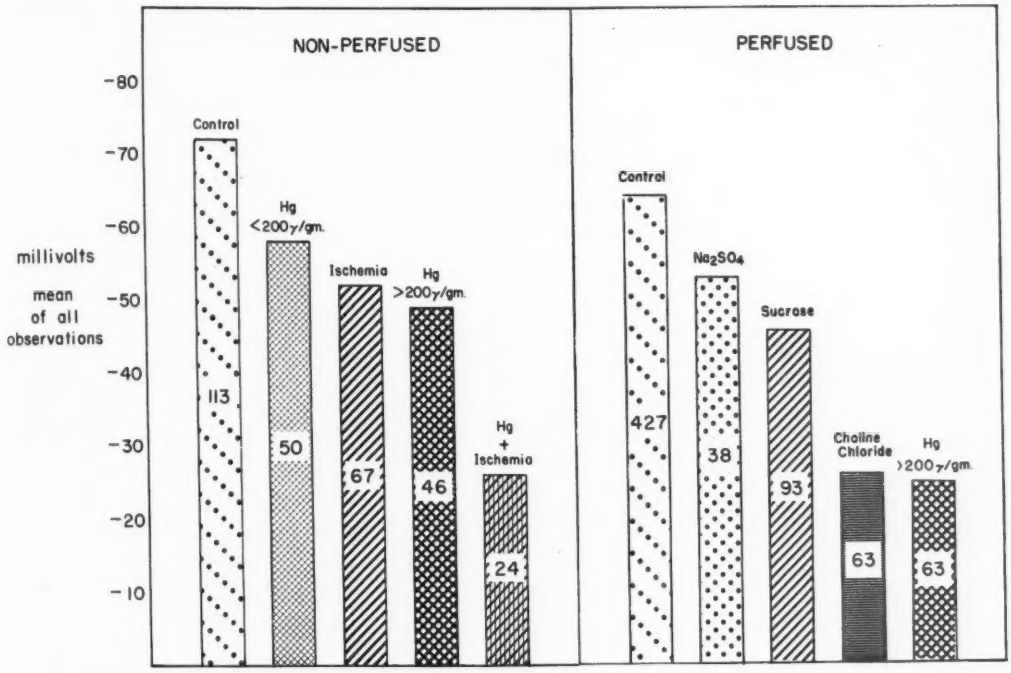

Figure 3

Summary of potential measurements done on single tubule cells. The numbers within the blocks indicate number of observations.[1, 4]

mammalian kidneys may offer an explanation. These authors observed considerable penetration of choline into renal cortical cells when slices were incubated in a low sodium–high choline chloride medium. It appears that choline penetrates kidney cells readily but cannot be extruded against a concentration gradient. These authors, as well as Whittam et al.,[11] described a loss of sodium and potassium from kidney slices under these conditions, since both are displaced in the intracellular fluid by choline. Since we have previously observed that the transmembrane potential of single tubule cells depends on the concentration gradient of potassium ions, a depolarization would be expected under conditions of perfusion in which most of the sodium ions have been replaced by choline.

Figure 4 gives a summary of our results on transtubular potential differences. On the left is the mean of 25 measurements in nonperfused kidneys. In all instances the tubule lumen was found to be negative with reference to the indifferent electrode, giving a mean of −20 mV (range: −8 to −30 mV). In contrast to the findings of Wilbrandt,[12] we have never found the inside of more distal sections of the nephron to be electrically positive to the outside. So far we have done only a few measurements across the distal tubule, all of which were approximately 15 mV more negative (i.e., mean −35 mV) than the mean value obtained from proximal transtubular measurements.

Similar results on normal transtubular potential differences were obtained by a number of other authors. Of interest are the observations of Kennedy,[13] Schatzmann et al.,[2] and of Whittembury,[3] confirming our results obtained across normal tubules and showing that potential differences can be maintained across perfused single tubules of Necturus. S. Solomon reported values ranging from −19 to −39 mV across the proximal tubule in the rat, and significantly higher values interpreted to

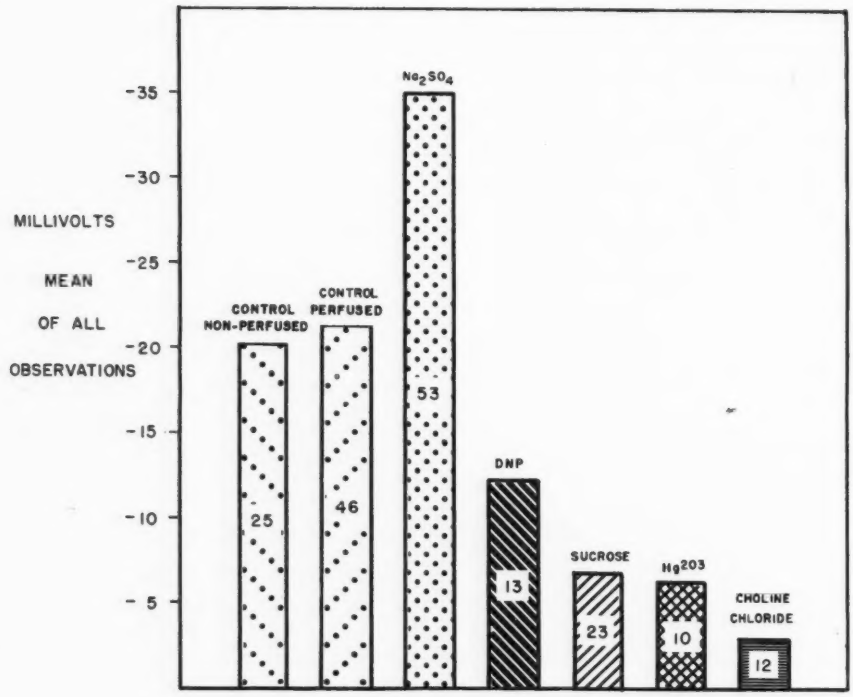

Figure 4

Summary of transtubular potential differences. The number of observations is indicated within the block diagrams.[1, 4] The value for DNP is that reported by Whittembury[3] and Solomon.[26]

have been measured across the distal tubule.[14] Rector also reports similar values to those of S. Solomon for proximal transtubular potential differences.[15]

From inspection of figure 4 it also can be seen that removal of sodium chloride in the perfusion fluid (perfusion with a buffered sucrose solution) drastically reduces the transtubular potential. Essentially the same effect is observed when choline chloride is used. The addition of Hg^{203} as Neohydrin[4] and of 2,4-dinitrophenol (2×10^{4} M) to the perfusion fluid when single tubules are perfused[3] is also effective in diminishing intratubular negativity. Sulfate-substitution results in enhancement of transtubular negativity.

An attempt will be made to present a concept of the origin of transcellular and transtubular potential differences. Our description of experimental results on a normal proximal tubule cell can be synthesized in terms of the diagram presented in figure 5. Certain key facts are significant in developing a theory of the origin of the observed electrical phenomena. First, the average transtubular potential difference is 21 mV, the tubule lumen being negative with respect to the peritubular fluid. The transcellular potential difference varies between 50 and 75 mV. In figure 5 the mean value of –64 mV is indicated. There exists, therefore, a net potential difference between interior of cell and lumen of 43 mV. On the other hand, a potential difference of 64 mV is maintained across the peritubular cell border. Secondly, renal tubular cells have a high concentration of potassium and a low concentration of sodium. Under the conditions of our experiment, an approximate value of

90 mEq./L. for potassium and 30 to 50 mEq./L. for sodium may be assumed. Thirdly, evidence will be presented to indicate that the proximal tubule cells actively transport sodium ions from tubule lumen to peritubular fluid, while the transtubular movement of chloride constitutes a passive process.[16] This is taken to indicate that the tubule cell must be permeable to these 2 ion species, a conclusion which is supported by a great number of observations made on kidney slices. By the same type of studies, considerable potassium permeability is also indicated.[17-23]

In the following, a hypothesis is presented which seems to be compatible with most observations. Let us consider the peritubular cell membrane first. The transcellular potential difference maintained across the peritubular cell membrane is close to that to be expected from the concentration gradient for potassium across this cell membrane. Accordingly, it behaves like a potassium diffusion potential. Additional and independent evidence that intracellular negativity is indeed closely related to the transcellular potassium gradient is given by the previously described variation of the peritubular cell-membrane potential as a function of the potassium concentration in the perfusion fluid. Taking a value of 90 mEq./L. for cell water as found in kidney samples of perfused preparations, and 4.5 mEq./L. for extracellular fluid, an average ratio of 20 obtains for K_i/K_o. Using the Nernst equation, $E = \frac{RT}{F} \ln \frac{K_i}{K_o}$, a ratio of 20 would correspond, at a temperature of 25 C., to a membrane potential of −77 mV. While we have observed values as high as this latter value, the majority of our measurements was lower (mean: −64 mV). There is no information available at present to account for this difference. As in other cell systems where similar deviations are well known, the possibility of cell injury at the site of impalement, of active potassium uptake[17] and of unequal activity coefficients[9] cannot be excluded. However, it seems more likely that the potassium selectivity of the peritubular, contraluminal membrane of the tubule cell is incomplete in such a manner as to permit some sodium ions to leak into the cell interior along their concentration gradient, thus partly discharging its intracellular negativity.

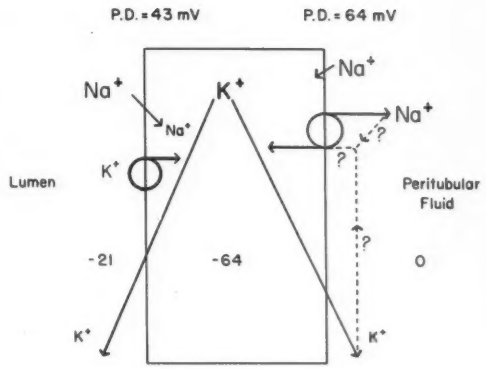

Figure 5

Model of renal tubular cell illustrating the hypothesis of the origin of renal tubular potential differences. The potential differences are the mean values observed in the perfused kidney of Necturus.[5] Note a potential difference of 64 mV maintained across the peritubular, and one of 43 mV across the tubular cell membrane. The length of the arrows indicates the relative permeabilities of sodium and potassium. The sodium extrusion mechanism is envisaged as being located at the cell membrane facing the peritubular fluid; potassium uptake is active at the luminal cell border.

The lower intracellular concentration of sodium and the finding that the proximal tubule normally pumps sodium out of the tubule lumen make it most reasonable to envisage the site of the active sodium extrusion mechanism at the peritubular cell border (see also [20]). This would explain a compartment of low intracellular sodium concentration interposed between tubular and peritubular fluid phase, into which sodium could diffuse passively along an electrochemical gradient.

It is, at present, uncertain whether this sodium extrusion mechanism (carrier) shows linkage with potassium (sodium-potassium exchange) or whether it incorporates an exchange diffusion component (sodium-sodium exchange). The relatively high rate of sodium

efflux from the tubule lumen at the observed low net transfer rates,[25, 26] makes the latter an attractive possibility.

Inspection of figure 5 indicates a potential difference of considerably smaller magnitude (43 mV) across the luminal cell membrane. Since the concentration of potassium is the same in tubular and peritubular fluid,[24] the different electrical potential gradients cannot be caused by different concentration gradients of potassium ions across the respective cell membranes. Since we have seen that the presence of sodium appears to be essential for the creation of the different potential gradients across the luminal and peritubular cell membranes, it seems logical to attribute an important role to this ion. We favor the view that the luminal cell membrane has a higher permeability to sodium ions than the peritubular cell side. This would permit positively charged sodium ions to diffuse along their electrochemical gradient into the cell and to shunt partially the intracellular negativity at this site.* Obviously, the effectiveness of this luminal depolarization also depends on the separation of sodium from its anions. If, for instance, chloride would enter the cell as readily as sodium, the effectiveness of the latter in reducing the transmembrane potential would be abolished.

A number of observations are consistent with the proposed cell model. Thus at the luminal cell side the observed transcellular potential is further removed from the potassium equilibrium potential than that at the peritubular cell membrane, indicating a higher degree of leakiness of this cell side to sodium. Another pertinent observation is the previously mentioned finding that complete removal of sodium from the perfusion fluid results in reduced transtubular potential differences. It should also be recalled in this connection that the rapid loss of sodium from kidneys exposed to a low sodium medium is quite consistent with an unusually high degree of sodium permeability of kidney cells. It seems logical to locate the main site of this sodium leakage at the luminal cell membrane and not at the peritubular cell side where the observed potential difference deviates much less from the potassium equilibrium potential and hence precludes such a high degree of permeability to sodium.*

Essentially then, the luminal transmembrane potential would constitute a potassium diffusion potential similar to that at the peritubular cell side but significantly modified by an opposing sodium diffusion potential caused by a higher degree of sodium permeability at the luminal cell surface.

It should be stressed that the contribution of the potassium diffusion potential at the luminal cell membrane is envisaged to be greater than that of the sodium diffusion potential, since the cell inside is negative with respect to the tubule lumen. Two experimental findings are consistent with this view: first, the previously described sensitivity of the luminal potential difference to changes in intratubular potassium concentration, and second, the relative insensitivity of the same potential difference to variation in the sodium concentration within the tubule. Thus, Whittembury,[3] in perfusion studies with choline chloride on single proximal tubules, showed

*If sodium and chloride move passively at the luminal cell border, the driving forces acting on these ions are not identical: sodium ions move along an electrical as well as along a chemical potential gradient, while the movement of chloride ions occurs against a sizable electrical gradient of 43 mV. In the absence of an active chloride transport system, the driving force for chloride movement would be furnished by the concentration gradient for chloride between tubule fluid and cell water.

*The observed reduction of transtubular and transcellular potential differences by mercurials is consistent with the observation made by others that mercurials inhibit proximal sodium chloride transport[27] in vivo, and in vitro lead to sodium gain and potassium loss in renal tissue. Inhibition of the active sodium extrusion mechanism[27-29] or increased passive sodium permeability[22] would explain the loss of cell polarization. Again, reduced transfer rate of positive charge out of the tubule would depress intratubular negativity. Furthermore, the depression of water and solute transfer across the proximal tubule of Necturus by 2, 4-dinitrophenol[3] and the simultaneous reduction of its transtubular potential difference[3, 26] indicate the relationship between sodium movement and intratubular negativity.

hat significant reduction of the transtubular otential difference occurred only when the ıtratubular sodium concentration had reached value of about 10 mEq./L. It should be ointed out that in terms of the total luminal otential difference, the degree of shunting ccomplished by sodium is relatively small nd that the potassium diffusion potential ecordingly dominates in its role as determiıant factor in the creation of the luminal poential difference.

Micropuncture studies performed by Bott[24] indicate that in Necturus there is a net reabsorption of potassium across the proximal tubule amounting to some 30 per cent of the filtered quantity. This movement occurs against an electrical gradient of 20 mV and in all probability constitutes active transport. Since the peritubular potential difference is much closer to the equilibrium potential generated by the concentration gradient for this ion than the luminal potential difference, it follows that, as Whittembury has pointed out,[3] the normal value for intracellular potassium is considerably in excess of that required for diffusional equilibrium across the luminal cell membrane. Stated differently, this smaller electrical gradient of 43 mV would be insufficient to prevent potassium leakage into the tubule lumen. A steady state can be maintained only if the deficiency in inward flux is compensated by an inward pumping mechanism which, for this purpose, must operate to give an inward potassium movement of the same magnitude as that due to an electrochemical potential of –20 mV.* Important considerations regarding the role of transcellular and transtubular potential differences, as far as the movement of hydrogen ions is concerned, have been presented by Pitts.[28]

In summary, it can be stated that the proximal tubule cell appears to possess, in principle, membrane elements for ionic movements which are similar to those in nerve, muscle, and other cells effecting a net transport of ions. These include an active sodium extrusion mechanism (possibly coupled with potassium uptake) about which, as in other systems, little is known, a passive potassium-selective diffusion element, and a sodium-selective diffusion element. In nerve and muscle, the sodium-selective element is activated only for milliseconds. In the frog-skin, Koefoed-Johnsen and Ussing[30, 31] showed that it is spatially completely separated from the potassium-permeable element. The proximal renal tubule cell seems to take an intermediate position in such a way that a potassium-selective diffusion element is dominant at both cell membranes, but the situation is modified by the presence of a significant sodium-selective diffusion element and possibly by active potassium uptake at that cell membrane which faces the tubule lumen.

Ion Flux Measurements on Single Renal Tubules

The rest of this presentation will be devoted to a discussion of some experimental results obtained by microperfusion of single proximal tubules in Necturus. The method of microperfusion of single tubules was originally introduced by A. N. Richards and Walker.[32] It has recently been modified, extended and successfully employed for the quantitative study of a number of problems of transtubular water and solute transfer by A. K. Solomon and his associates.[33] Briefly, it consists of the deposition between oil of a small amount of fluid of known composition in a tubular segment, its withdrawal after a known time interval, and comparison with its original composition.

These methods have recently been employed to study some aspects of sodium and chloride transport across the proximal tubule.[16, 25] Several lines of evidence may be cited in favor of active proximal sodium transport in Necturus. First, net sodium movement occurs against an electrical potential gradient of some 20 mV.[1, 2, 25, 26] Second, as Windhager et al. have shown,[34] sodium movement also occurs against a sizable concentration gradient when a mixture of sodium chloride and manni-

*It is to be expected that this process of active otassium uptake at the luminal cell border also conributes to the lowering of the luminal transcellular otential difference, since such carrier-mediated potasium influx is opposed to the potential-generating outard movement of potassium.

Table 1

*Flux Measurements and Flux Ratios of Chloride in the Perfused Proximal Tubule of Necturus**

Exp. no.	CP/IP Inulin	CP/IP Cl^{36}	Efflux $\mu\mu$Eq.	Influx Cl cm.$^{-2}$	Net flux sec.$^{-1}$	M_o/M_1
1	1.18	0.164	370	338	32	1.10
2	1.14	0.099	463	436	27	1.06
3	1.32	0.210	325	275	50	1.18
4	1.67	0.074	497	415	82	1.20
5	1.19	0.100	457	425	32	1.08
6	1.25	0.486	172	131	41	1.31
7	1.40	0.109	432	374	58	1.16
8	1.36	0.430	202	148	54	1.36
9	1.19	0.409	200	168	32	1.19
Means			346	301	45	1.18±0.1

*Flux measurements CP/IP refer to the ratio C^{14} inulin concentration in collected perfusate/C^{14} concentration in injected perfusate. The same applies for Cl^{36} CP/IP ratios.

tol is deposited within the lumen of the proximal tubule. Finally, experiments carried out by Whittembury and his associates[35] have indicated such a permeability of the proximal tubule wall to water as to preclude the normally occurring colloid osmotic pressure difference from playing a significant role in proximal fluid reabsorption in Necturus.

As far as the reabsorptive chloride movement is concerned, it appears possible that it constitutes a passive process downhill along a transtubular electrical potential gradient. However, such downhill movement per se does not exclude the possibility of active chloride transport. A means of quantitating the relationship between electrochemical potential gradient and ion movement was given independently by Teorell[36] and Ussing.[37] The advantage of their approach consists in the fact that all driving forces acting upon an ion species under consideration can be measured and related to observed ion fluxes. In our specific instance of chloride transfer across the proximal tubule, we were particularly interested in whether the known electrical asymmetry could quantitatively account for the observed chloride fluxes, or whether an additional driving force had to be invoked.

In order to obtain direct information about bidirectional chloride movement, perfusion studies on single proximal tubules of Necturus were performed in collaboration with Dr. Windhager.[16] Results are summarized in table 1. Net water movement across the proximal tubular wall was estimated by the use of C^{14}-labeled inulin; the fact that all ratios exceed unity indicates significant fluid reabsorption. Chloride[36] was added to the perfusion fluid and its radioactivity compared with that of the collected fluid (column 3). In a number of experiments, chemical chloride experiments were also done on the collected perfusate and the plasma, using Ramsay's second electrometric titration method.[38] We did not find a significant difference of chloride concentrations between collected perfusate and plasma. For the calculation of chloride efflux we have used, in essence, an equation developed by Curran and Solomon.[39] We have estimated the net movement of chloride from the chemical chloride concentration and the amount of fluid reabsorbed, the latter being measured by the inulin concentration ratios. Data on influx were derived from the measured efflux and net flux values.

The efflux values found averaged 346 $\mu\mu$Eq. cm.$^{-2}$ sec.$^{-1}$, those for influx 301 $\mu\mu$Eq. cm.$^{-2}$ sec.$^{-1}$. There is apparently a considerable variability between individual tubules, yet the ratio of efflux/influx is fairly constant. In a similar series of experiments, reported by Oken et al.,[25] a value of 266 $\mu\mu$Eq. cm.$^{-2}$ sec.$^{-1}$ was found for sodium efflux and 204 $\mu\mu$Eq. cm.$^{-2}$ sec.$^{-1}$ for sodium influx. Both these results indicate that net movement of chloride and sodium constitutes only a relatively small fraction of the total efflux. Stated differently, it means that proximal tubular rate of turnover for sodium and chloride is quite high.

Using a mean transtubular potential difference of 20 mV, as found by Schatzmann et al.,[2] by us,[1, 4] and by Whittembury,[3] across normal and perfused proximal tubules of Necturus, a calculated flux ratio of 2.14 is obtained (fig. 6). The fact that the calculated flux ratio is greater than the observed one of 1.18 indicates that the usual driving force of 20 mV readily accounts for the measured flux

$$\frac{M_o}{M_i} = \frac{c_i}{c_o} e^{\frac{ZF}{RT} \cdot E}$$

OBSERVED: $\frac{M_o}{M_i} = 1.18$

CALCULATED: $\frac{M_o}{M_i} = 2.14$

Figure 6

Summary of observed and calculated flux ratios assuming a transtubular potential difference of −20 mV. For further explanation, see text.

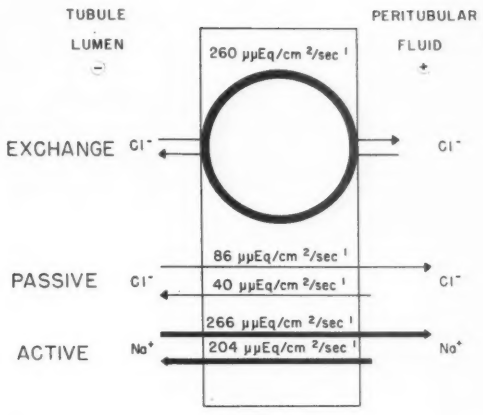

Figure 7

Mean of flux measurements across single proximal tubules of Necturus. The values for chloride[16] are fractioned into a passive and exchange diffusion component. Values for sodium fluxes are those of Oken et al.[25]

asymmetry. We interpret this finding as evidence for passive chloride movement across the proximal tubule along the electrical potential gradient.

Since the observed flux asymmetry is smaller than that to be expected on the basis of a 20 mV potential difference, it seems possible that part of the observed chloride movement is due to exchange diffusion. Levi and Ussing[40] first suggested such a mechanism, the essence of which is that part of an observed ion movement is independent of the electrical field and equal in both directions, thus not affecting net transport. This would essentially apply to that part of the chloride which moves in a bound, electrically uncharged form and is, consequently, not affected by the 20 mV driving potential. Similar situations for sodium and chloride appear to apply for a variety of biologic membranes, such as gastric mucosa,[41, 42] the large intestine of the frog,[43] muscle,[44] and erythrocyte.[45]

A schematic representation of such a possible partition of chloride fluxes is shown in figure 7. The diagram also includes for comparison the values for bidirectional sodium fluxes as reported by Oken et al.[25] A tubular negativity is indicated at the left. This 20 mV constitutes the driving force for that portion of free chloride movement which is passive and which is represented by the straight arrows in the lower part of the graph. Cooperstein and Hogben[43] have given a similar schema for the large intestine of the frog, where flux discrepancies similar to those to be expected from the spontaneous potential and free chloride movement were observed. Following their calculation, our data would indicate that the larger part of the chloride movement occurs by this latter mode of transfer. This part of the chloride movement by exchange diffusion, being independent of the electrical potential, is visualized in the upper part of the diagram by the large circle, signifying no net movement. The data presented do not allow us to estimate the contribution of solvent drag on chloride movement. Additional data will be needed to settle this point which does not, however, materially affect the main conclusion of a passive nature of transtubular chloride movement.

In summary, experimental technics for the measurement of stable electrical potential differences across single renal tubular cells

and across single tubules are described. They indicate that the inside of the tubule lumen is normally electrically negative to the outside and that the interior of the cell is negative with respect to both the peritubular fluid and the tubular lumen. It is suggested that these potential differences are created by the formation, within the cell, of an area rich in potassium and relatively low in sodium. An active sodium extrusion mechanism is located at the peritubular cell membrane, the latter being relatively less permeable to sodium than the cell membrane facing the tubule lumen. It is further suggested that the passive diffusion of sodium at a higher rate than chloride, and the active potassium uptake from the tubule lumen into the cell, partially shunt the potential difference across the cell and at the same time render the inside of the tubule electrically negative. Since the net reabsorptive movement of sodium across the proximal tubule occurs against an electrical gradient, it is an active process. The electrical gradient caused by this extrusion of positively charged sodium ions is oriented in such a way as to account quantitatively for passive reabsorptive movement of chloride out of the tubule. Perfusion studies of single proximal tubules demonstrate a high rate of turnover of both sodium and chloride, indicating that the net movement of these ions is only a relatively small fraction of the unidirectional fluxes.

Acknowledgment

I am greatly indebted to Dr. E. E. Windhager for much helpful discussion of many aspects of this presentation, and for his introducing me to the technic of perfusion of single tubules. I am also grateful to Dr. P. A. Bott in whose laboratory I became acquainted with micropuncture technics, and to Dr. R. F. Pitts for constant encouragement and support. It is also a pleasure to acknowledge the permission given by Drs. G. Whittembury, D. E. Oken and A. K. Solomon to cite some of their unpublished work.

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Ion Exchange Mechanisms in the Nephron

By ROBERT W. BERLINER, M.D.

Accumulating evidence favors the view that in biologic systems, the active transport of strong electrolytes involves coupled exchanges of ions, usually of sodium for potassium. In the present paper, the author considers the renal exchange of potassium, sodium and hydrogen in this light, and reviews current concepts concerning the mechanisms involved in the secretion of these ions by the kidney. He indicates that new investigative technics will be required to establish that the kidney behaves with respect to coupled ion exchanges as do nerves, red blood cells and frog skin. He concludes by cautioning against extrapolation of experimental results from one species to another.

THE TITLE "Ion Exchange Mechanisms in the Nephron" permits a more or less wide range of subject matter depending upon the interpretation of the phrase "ion exchange mechanisms." This presentation will be concerned with a few aspects of the processes involved in the excretion of acid and potassium, which are the processes which have been shown to result in the replacement of 1 ion in the urine by another. However, as knowledge increases concerning the transport of strong electrolytes generally, the evidence increasingly favors the concept that all such active-transport processes involve coupled exchanges—usually of sodium for potassium. At least such exchanges appear to be implicated in nerve,[1] in red blood cell[2] and in frog skin,[3] and it seems reasonable to expect that when adequate investigative technics have been devised for the more definitive study of renal tubular processes, similar mechanisms will be encountered. Indeed, the carrier mechanism now widely adopted as the model for ion transport implies ion exchange.[4]

With respect to potassium excretion, no attempt will be made to review the entire field; certain aspects related to relatively recent work will be considered. One of these aspects concerns the site and extent of potassium reabsorption in the tubule. While very little is known about the mechanism concerned in this process and it is not, strictly speaking, one which can be identified with the ion exchange processes, some knowledge of the reabsorptive process is essential for the evaluation of the secretory sodium-potassium exchange, since only the difference between reabsorption and secretion can be measured. To quantify secretion, it is first necessary to assign some value to reabsorption. Gilman was the first to propose that reabsorption of potassium was equal to the filtration of potassium—that is, that the filtered potassium was completely reabsorbed before the potassium destined for excretion was added by secretion.[5] At the time, 10 or 12 years ago, this seemed a rather unlikely possibility; but as time has passed and more data have been accumulated, it first became useful to adopt this as a working hypothesis,[6] and then as a view for which there is a considerable body of experimental evidence.

The evidence for this view is based largely on 2 phenomena: first, the dependence of the excretion of potassium on the excretion of sodium and second, the lack of relationship between the glomerular filtration rate and the excretion of potassium when the excretion of sodium is maintained. Although earlier data illustrating the dependence of the excretion of potassium on the excretion of sodium will be considered later, the most striking demonstration of this phenomenon is to be seen in stop-flow studies.

Figure 1, taken from some recent studies by Jaenike and Berliner,[7] shows the usual pattern of electrolyte concentrations in stop-flow studies. In this particular instance the first few samples collected, shown at the left of the figure, differ from the usual because in

From the National Heart Institute, National Institutes of Health, Bethesda, Md.

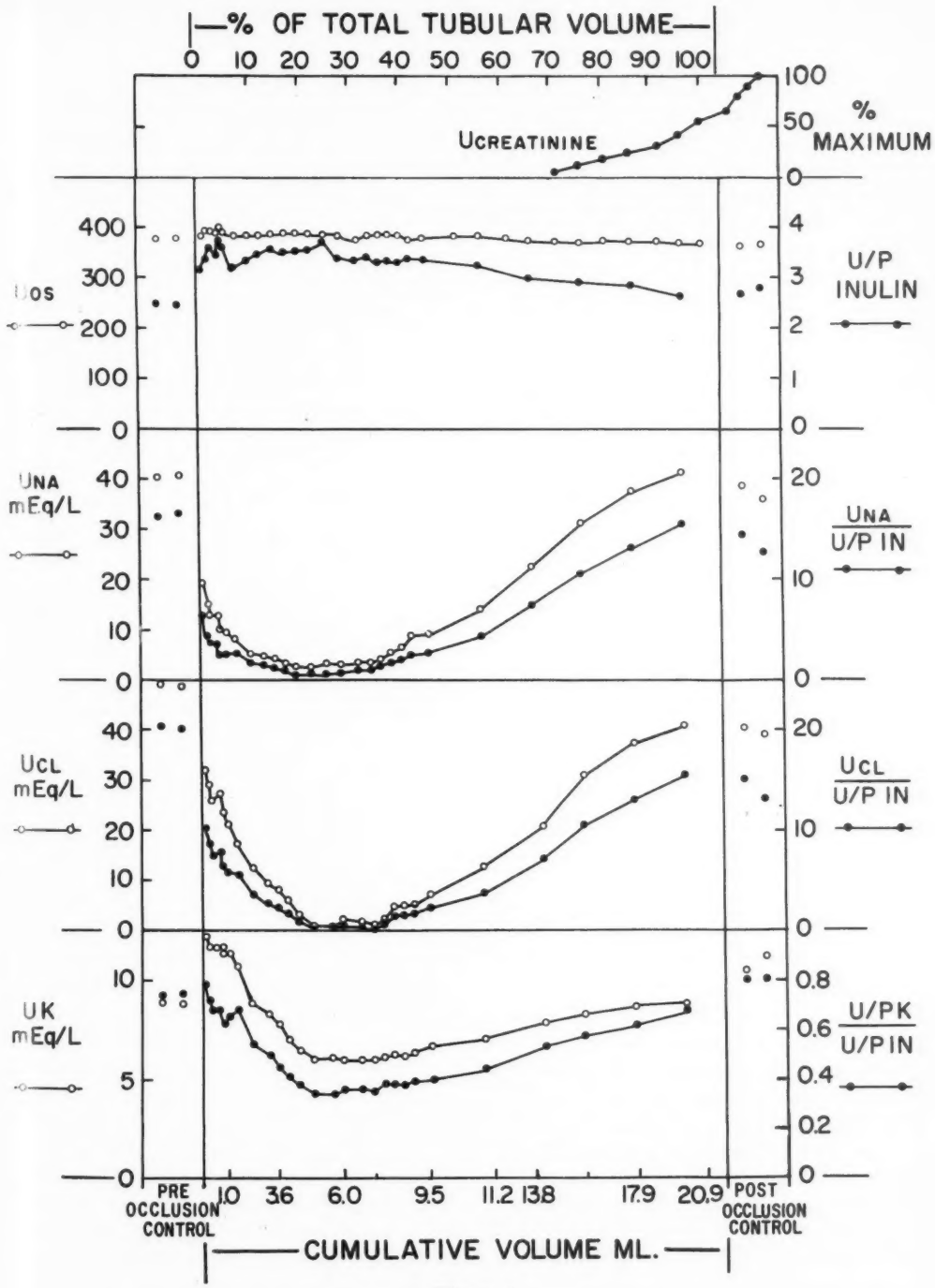

Figure 1

Stop-flow pattern obtained during mannitol diuresis in the dog. The dead space had been filled with mineral oil.[7] First specimens collected appear at the left of the figure. (Republished by permission of the Journal of Clinical Investigation.[7])

these studies the pelvic dead space was filled with oil. The first samples, therefore, differ from free flow because they are derived from the tubule system and have been modified during the period of stop-flow. This is not important for present purposes, but will explain the different appearance of these data from the figures of Vander, Sullivan, Malvin, and Wilde.[8, 10] With this exception, the pattern of potassium concentration seen here does not differ significantly from that previously published by others.[8, 9] The concentration of potassium shows a minimum in samples which, from their very low concentrations of sodium and chloride, are identified as having sojourned in distal tubules during the period of stop-flow. This low potassium concentration might be due to reabsorption of potassium at the indicated site. However, the samples from that site must traverse the remainder of the tubule system before they are collected and the more distal parts of the tubule certainly can and do secrete potassium.[9, 10] The low concentrations of potassium might also be interpreted as being due to failure to secrete potassium into those particular samples. There is a logical explanation for this possibility in that they contain virtually no sodium to be exchanged for potassium. These 2 explanations have been distinguished in experiments by Walker and Cooke and their associates at Johns Hopkins.[11] The results of these experiments very clearly indicate that the latter interpretation is correct, that is, that the low potassium concentrations are due to inability of the more distal parts of the tubules to add potassium to the virtually sodium-free distal tubule samples. The experiments were done using a modification of the stop-flow technic devised by Murdaugh and Robinson.[12] This modification consists of interrupting the stop-flow at the end of 4 minutes to permit samples from 1 site to move down to another. Flow is then stopped for an additional 4 minutes, following which the usual type of collection is made. If, during the interruption of stop-flow, about 3 or 4 ml. is permitted to escape from the tubules, most of the high potassium samples leave the tubules and the low sodium–low potassium samples are moved into the region in which potassium concentration was previously high. When the pattern from this second occlusion is examined, there is no distal potassium peak. Furthermore, if the secretion of potassium is markedly enhanced by the administration of the carbonic anhydrase inhibitor, acetazoleamide, the marked distal peak observed in the initial collection is still obliterated in the second part of the interrupted stop-flow. On the other hand, if chlorothiazide is used so that the minimal concentration of sodium in distal tubule samples is much higher, the potassium peak, although somewhat reduced, is still present. The question arises whether the failure to observe a potassium peak during the second occlusion does not stem from either damage to, or exhaustion of, the secretory mechanism during the prolonged period of stop-flow. This problem was tested by permitting 8 to 10 ml., rather than 3 to 4 ml., to escape between occlusions so that the low sodium samples could move out of the tubules; the results indicate that under this circumstance the potassium peak in the second occlusion was indistinguishable from the first.

Several conclusions can be drawn from these observations: (1) potassium can be secreted only into fluids which initially contain sodium; (2) the potassium minimum in stop-flow experiments owes its localization primarily to the absence of sodium in the same samples. Had the urine, in the moments preceding stop-flow, arrived at this site already freed of potassium but not of sodium, the result would be exactly that observed. Both more distal and more proximal samples would contain higher concentrations of sodium and therefore gain potassium in passing the secretory site after reinstatement of flow, while those around the sodium concentration minimum would remain free of potassium. It can be concluded, concerning the site of potassium reabsorption, that it *may* be at the region which is marked by the low concentrations of potassium in stop-flow patterns, but it may also be anywhere proximal to it. Furthermore, the concentration of potassium in these

low potassium samples reveals nothing about how much potassium was left in them by the reabsorptive process, since they subsequently gain potassium while passing the secretory site. If some intervention, such as the administration of chlorothiazide or the infusion of sulfate, results in a higher concentration of potassium in the low potassium samples, it does not warrant the conclusion that the reabsorption of potassium has been inhibited;[8, 13] this reservation is particularly pertinent if the intervention increases the concentration of sodium in these samples, in which case it will enhance their capacity to accept potassium after flow is reinstituted. As a matter of fact, data to be presented shortly, much of it derived after just such interventions, strongly support the view that even under these conditions all of the filtered potassium is reabsorbed.

It is apparent from the data already considered that in some tubule segments all, or nearly all, the potassium can be reabsorbed. However, this reabsorption, which occurred during the relatively prolonged stasis of stop-flow, might not occur during free flow. The data of Davidson, Levinsky, and Berliner[14] show that the same extensive reabsorption of potassium does occur in free flow. The pertinent experiments involved the separate collection of urine from each kidney of the trained, unanesthetized dog which had been previously prepared by a bladder-splitting procedure.[15] At appropriate times the filtration rate in the right kidney was reduced by inflation of a cuff previously placed around the right renal artery and connected to the outside by a fine polyethylene catheter.[16] This made it possible to use the left kidney as a control for the right and thus to compensate for changes with time in the excretion of potassium from the control kidney. When no attempt was made to provide a high rate of sodium excretion, reduction of the filtration rate produced a sharp drop in sodium excretion and with it a marked, although lesser, drop in potassium excretion. This is illustrated in figure 2. Since sodium excretion frequently fell to quite low levels, the results were consistent with the interpretation that the observed effect was due, not primarily to the change in filtered potassium, but to the decrease in the sodium available for exchange with potassium in the more distal parts of the nephron.

Accordingly, measures of various sorts were taken to increase sodium excretion to such an extent that a high rate of sodium excretion was maintained even when glomerular filtration was reduced. The procedures which were used were the administration of the mercurial diuretic, salyrgan, the carbonic anhydrase inhibitor, acetazoleamide, and the poorly reabsorbed anion, sulfate, in the form of sodium sulfate. The result obtained, as shown in figure 3, was independent of the procedure used to maintain sodium excretion. Reduction of glomerular filtration rate by up to 35 per cent was entirely without effect on the excretion of potassium, although sodium excretion fell to an extent equal to or greater than the reduction of filtration rate. The excretion of potassium was maintained at the same level as in the control kidney whether that level represented only 15 to 20 per cent of the concurrent rate of filtration of potassium at the glomeruli, as was frequently the case when salyrgan was administered, or, as was usual when acetazoleamide was used, when the rate of excretion of potassium exceeded the rate at which it was filtered. The only reasonable interpretation of these data is that the filtered potassium makes no appreciable contribution to that excreted in the urine. If only a few per cent of the filtered potassium were excreted, the total excretion of potassium should have been detectably modified by the change in filtration rate. It should also be noted that the data can be explained only if the reabsorption precedes the secretion.

The data derived from micropuncture studies are the only ones which pertain directly to the localization of the site of potassium reabsorption. These data are conflicting. Wirz and Bott,[17] in the only micropuncture study of potassium in the mammal, found that in the rat the concentration of potassium fell markedly in the first half of the proximal tubule. At about the same time, Bott also found that the concentration of potassium was

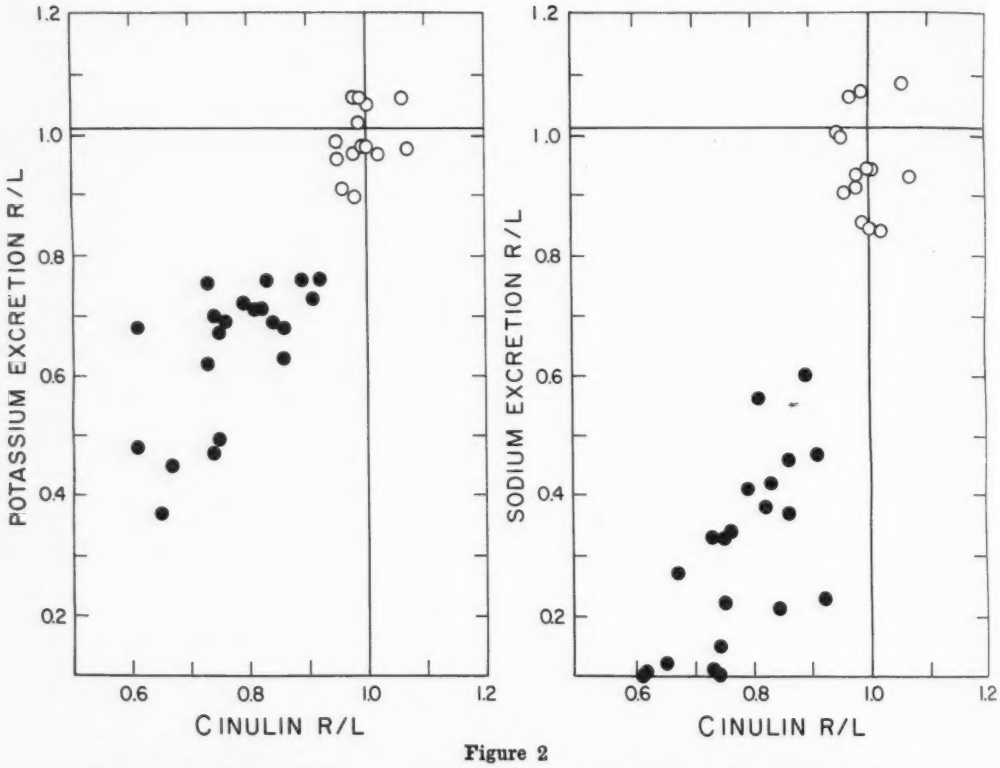

Figure 2

Potassium (left) *and sodium excretion* (right) *during reduction of the glomerular filtration rate in dogs in which no attempt was made to maintain sodium excretion.* Ordinate: *ratio of potassium or sodium excretion of the right (experimental) kidney to that of the left (control) kidney;* Abscissa: *ratio of inulin clearances right kidney to left kidney.* Open symbols: *control periods (before inflation of cuff on right renal artery);* Solid symbols: *experimental periods.* (*Republished by permission of the Journal of Clinical Investigation.*[14])

markedly reduced in the proximal tubule of Necturus.[18] But, subsequently, using different and probably more reliable methods, Bott did not find an appreciable reduction in the concentration of potassium in the proximal tubule of Necturus.[19] Nevertheless, in view of the opposing electrical potential,[20] even the maintenance of an unchanged concentration requires active transport of potassium (or a very considerable solvent drag effect);[21] otherwise, if active transport were not involved, the potassium concentration should rise to almost twice that in plasma in order for the chemical gradient to balance the electrical gradient. It may be, then, that reabsorption of potassium is completed in some more distal segment, but for the reasons stated earlier, the site cannot be identified by the stop-flow technic.

The recent observations of Hilger, Klümper and Ullrich[22] based on microcatheterization of the hamster papilla, have suggested, although they did not unequivocally establish, that potassium secretion occurs in the collecting ducts. Data from the stop-flow experiments of Jaenike and Berliner[7] suggest that the same is true in the dog. However, it should be pointed out that the secretion of potassium cannot be confined to the collecting ducts but must, in large measure, be contributed by cortical portions of the nephron. It is not difficult to produce, and maintain over extended pe-

riods, potassium clearances twice the rate of glomerular filtration, which in the dog would be about half the renal plasma flow. Since, barring synthesis by the kidney, no substance can long be cleared at a rate exceeding the blood flow, this means that the potassium secretory site must receive a flow of blood which can supply the requisite potassium. Even assuming that there is no reabsorption of the filtered potassium, and disregarding the probable relative exclusion of potassium from the medulla by the countercurrent flow of the blood, the most generous estimates of medullary blood flow would not include enough potassium to supply the secreted potassium. Similar considerations probably also apply to the availability of ammonia precursors to the collecting ducts, but the necessary data for the calculation are less easily obtained.

There is little information concerning the mechanism by which the potassium-sodium exchange is accomplished. It appears not unlikely that potassium-sodium exchange underlies the sodium extrusion mechanism which characterizes virtually all animal cells. However, except for the red blood cell, potassium seems to be close to electrochemical equilibrium[23] in those cells that have been studied. This may be due to a high permeability to potassium which enables potassium to be distributed in such a way that there is little departure from an electrochemical potential gradient of zero. The known low permeability of the red blood cell to cations will adequately explain its departure from the behavior of other cell types. In the case of membranes such as frog skin and renal tubule epithelium, which perform oriented transport, there is an additional requirement for a differential permeability of the 2 surfaces of the cell facing the exterior and interior of the body. This is essentially the model of Ussing and Koefoed-Johnsen,[3] in which potassium is close to electrochemical equilibrium across the inward-facing surface of the cell, but not across the outward-facing surface which has a low permeability to potassium. The common sensitivity of all the sodium-potassium transport mechanisms, including that of the renal tubules, to strophanthidin and other cardiotonic steroids suggests, as pointed out by Orloff and Burg,[24] that a similar sodium-potassium exchange process at the basal surface of the tubule epithelial cells underlies the monovalent electrolyte transport systems of the renal tubules. Whether or not any additional process is required to explain the secretion of potassium into the urine is uncertain, since the value of the electrical potential gradient between tubule lumen and peritubular fluid has not been measured at the right place and under the right conditions. However, the concentration ratio (urine to interstitial fluid) for potassium probably rarely exceeds 20 or 30 and the required 80 to 100 mV may well be present at these times. It should be noted that so long as the cell membranes are permeable to chloride, and chloride is present in appreciable concentration in the lumen, there would be a continuous flow of chloride from lumen to the peritubular fluid. This current of chloride ion would short-circuit the potential, keeping it relatively low and tending to suppress the accumulation of potassium in the lumen.

Although there is some virtue in simple hypotheses, the fact that a relatively simple model to explain net sodium-potassium exchange can be devised does not mean that nothing more is involved. One circumstance that makes this model seem incomplete arises in consideration of the problem of acidification. The ratio of hydrogen ion concentration in urine to that in plasma may reach 1,000 or more and a transtubular potential of 180 mV or more would be required to establish such a gradient passively. This suggests, although it does not prove, the existence of some more specific exchange mechanism, presumably at the luminal face of the cell as proposed in the model of Pitts[9] (fig. 4). The addition of such an exchanger to the model would account for the exchange of potassium for sodium and explain the competitive relationship between potassium and hydrogen ion,[6] a phenomenon which is a little more difficult to fit in with the simple model of a single pump.

In the further consideration of hydrogen ion exchange, a problem of definition exists. It has been fairly generally accepted that the

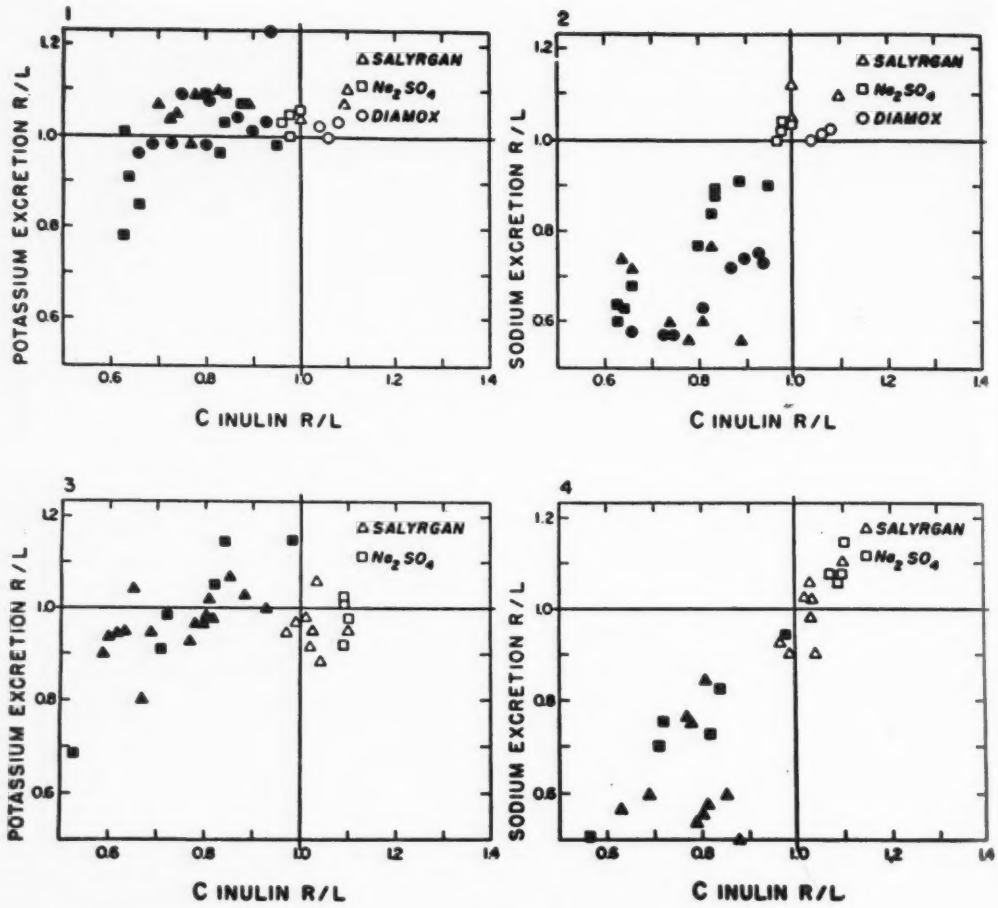

Figure 3

Potassium (left) *and sodium excretion* (right) *during reduction of the glomerular filtration rate in dogs with maintained sodium excretion from the experimental kidney. See legend figure 2. (Republished by permission of the Journal of Clinical Investigation.[14])*

reabsorption of bicarbonate and the formation of titratable acid are effected by the secretion of hydrogen ion in exchange for sodium (fig. 5).[4, 25, 26] The secreted hydrogen ion is assumed to combine with bicarbonate to yield carbonic acid which in turn yields CO_2 and water which may diffuse from the urine. There has been little direct evidence to support this view, but because this unitary hypothesis is simplest and there is no evidence to the contrary, it seems expedient to retain it. This view has, indeed, been somewhat strengthened by the recent findings of Gottschalk and Giebisch and their respective associates[27, 28] that the pH of proximal tubule urine in the rat does not, as had long been considered to be the case, remain the same as that of plasma. Instead, it is lowered appreciably. Thus, one assumed difference between bicarbonate reabsorption in the proximal, and acidification in the distal tubule has been largely eliminated from consideration. One little-recognized consequence of the concept that bicarbonate reabsorption is effected by

hydrogen-sodium exchange is that considerably more hydrogen ion is secreted in the proximal tubule where most of the bicarbonate is reabsorbed than in the more distal regions where the extremes of acidity are reached. This consequence is made all the more one-sided by the observation that bicarbonate concentration, as well as volume flow, is reduced in the proximal tubule.

One other very interesting consequence which may make possible the experimental verification of the hypothesis has been pointed out by Walser and Mudge.[29] It can be calculated that if proximal bicarbonate reabsorption involves the formation of carbonic acid and its subsequent dehydration to carbon dioxide, as would be the case with hydrogen ion secretion, the latter step can proceed at a rate equal to the rate of bicarbonate reabsorption only if: (1) there is carbonic anhydrase in the surface of the proximal tubule cells so that the reaction of carbonic acid to CO_2 and water in the lumen is catalyzed; or (2) there is appreciable accumulation of carbonic acid in the fluid in the tubule—enough to bring the pH down approximately 1 pH unit below its equilibrium value. Unfortunately, the type of pH measurement which has been made, since it involves withdrawal or at least isolation of an element of fluid, does not exclude (although it does make it improbable) the possibility that the pH of the fluid *in situ* is as low as required by hydrogen ion secretion in the absence of carbonic anhydrase. In the interval required to do the analysis, uncatalyzed dehydration of carbonic acid might have raised the pH to the observed value.

The third effect of hydrogen-sodium exchange is the accumulation of ammonia in the acidified urine. The process is believed to involve the formation of ammonia from appropriate precursors in the cell of the renal tubule and the diffusion of the nonionic ammonia, that is NH_3, into the urine; in the urine it combines with hydrogen ion to form ammonium ion to which the tubule cell is presumed to be relatively impermeable. There are those who prefer to believe that the excretion of ammonium ion involves direct exchange of ammonium and sodium ions.[30, 31] This view, however, fails to account for one of the most striking features of ammonia excretion (at least in man and in the dog)—its dependence upon the pH of the urine.[32, 33] The argument advanced that glutamine hydrolysis yields, at the pH within cells, glutamate and ammonium ions[31] is without cogency since even if free ammonia is formed, it must be considered to be instantaneously in equilibrium with its ionic form which greatly predominates at all physiologic pH's. This has, however, little bearing on the form in which the ammonia crosses the cell membrane. With regard to the latter question there is a very considerable body of evidence indicating that cell membranes are very much more permeable to nonpolar, nonionized substances than to their ions.

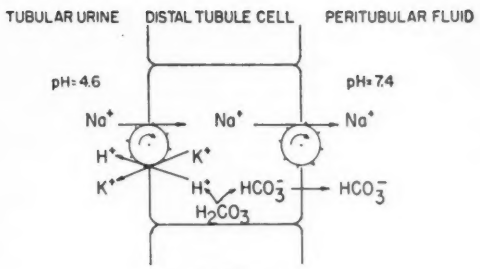

Figure 4

Schematic representation of distal tubule exchange mechanisms. (Republished by permission of the American Journal of Medicine.[9])

In closing, it seems warranted to mention a need for some caution in accepting data obtained in rats as quantitatively applicable to all mammals. While it is unlikely that similar mechanisms in different species differ in their qualitative nature, and indeed we have reason to hope that the fundamental similarities of transport mechanisms extend from tissue to tissue as well as from one animal type to another, nevertheless, it may be misleading to extend quantitative considerations from one to another. This is well illustrated in some of the disagreements concerning the determinants of ammonia excretion. These disagreements have certain of the features of the old story about the blind men and the elephant.

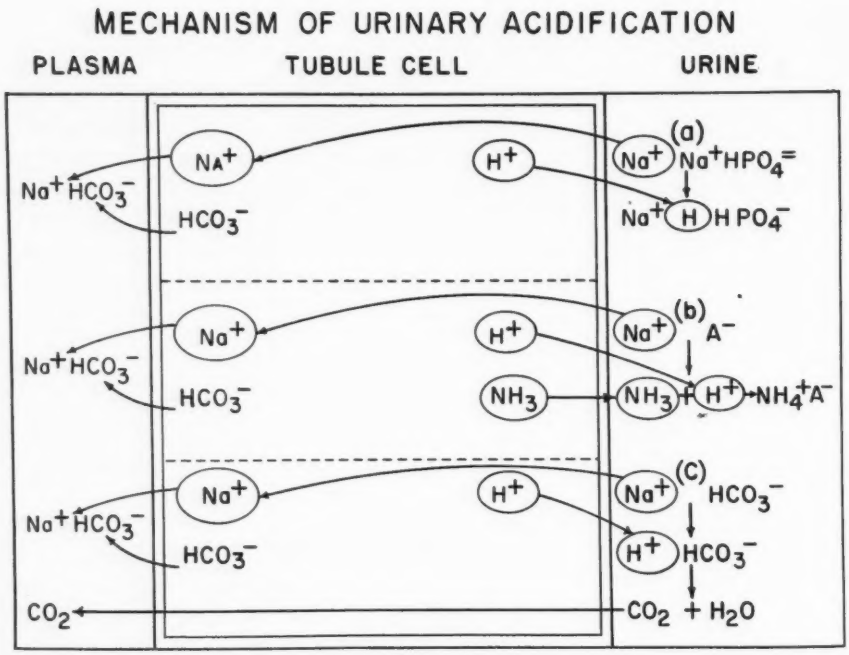

Figure 5

Schematic representation of hydrogen-sodium exchange and its effects on the composition of fluid in the tubule lumen.

Those who have studied ammonia excretion in the rat have been impressed with the marked and easily demonstrable increases in the glutaminase activity of the renal cortex, which over any relatively extended period are highly correlated with the rate of ammonia excretion.[30, 34] However, adaptation to acidosis in the dog is not associated with any such change in glutaminase activity,[35] and in this species the relationship of ammonia excretion to the pH of the urine is much more striking and reproducible.[33, 37] Furthermore, the increase in ammonia excretion, which occurs in rats along with an increase in glutaminase activity in response to carbonic anhydrase inhibitors, is absent in both dog[36] and man.

It is important, therefore, to extend the basis of our knowledge over as wide a range as possible and, particularly with regard to quantitative aspects, to hold some reservations, until our investigations have been extended to the specific circumstances concerning which we wish to draw conclusions.

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Stop-Flow Studies on Ion and Water Reabsorption in the Dog

By RICHARD L. MALVIN, PH.D., AND WALTER S. WILDE, PH.D.

The stop-flow method has been used to study various aspects of renal tubular transport mechanisms. According to this method, aldosterone promotes the tubular reabsorption of sodium in the distal tubule. The method also indicates that the secretion of potassium, hydrogen and ammonium occurs in a very distal portion of the nephron. The substitution of anions such as ferrocyanide, sulfate and phosphate for chloride apparently increases the area of the nephron involved in the secretion of hydrogen and potassium. The stop-flow experiments support other evidence that the rate of flow of urine through the loops of Henle affects the countercurrent multiplier system which, in turn, determines the ability of the kidneys to elaborate a concentrated urine.

IT HAS been known for many years that the kidney reabsorbs a large part of the salts and water filtered at the glomerulus. However, only in recent years have some of the mechanisms controlling secretion and reabsorption been elucidated. The micropuncture technic as developed by Richards[1] has been extremely useful in this respect, since it allows direct analysis of tubular urine. The stop-flow method, although not as precise as micropuncture, has also lent itself well to renal transport studies.

For the past 2 years, the authors, in collaboration with Drs. Lawrence Sullivan, Arthur Vander and Peter Abbrecht, have used the stop-flow technic extensively as a tool for investigating the transport of salt and water in the nephron. Because of the limitations of time, we have arbitrarily selected 3 areas of our research for discussion at this symposium: (1) the effect of aldosterone on the transport of sodium; (2) the effect of large impermeant anions on the transport of potassium; (3) the effect of osmotic diuresis on the renal concentrating mechanism.

Aldosterone

Recently,[2] using the stop-flow technic, we demonstrated a distal site of action of aldosterone. The dashed curve in figure 1 illustrates a typical pattern of the concentration of sodium from a stop-flow experiment in a normal dog. The sodium concentration of free flow urine collected immediately before occlusion was 63 mM per liter. As distal fluid entered the collector, the concentration of sodium fell to a low of 5 mM per liter. As fluid from the loops and proximal tubules entered the collector, the concentrations of sodium rose to reach a plateau at the original free flow concentration. The peak concentration of para-aminohippurate (PAH), not shown in this figure, indicated the best stop-flow proximal sample to be at 10 ml.

In contrast to the normal dog, the lowest concentration achieved by the distal tubule of the adrenalectomized dog was only 24 mM per liter. The distal tubule was apparently unable to reduce the concentration of sodium to the minimum value achieved in normal dogs during stop-flow.

Occasionally, normally low concentrations of sodium were obtained from the distal tubular urine of the adrenalectomized animal. This seemed to occur only when the concentration of sodium in plasma was exceedingly low. The following series of experiments was designed, therefore, to study the relationship between the concentrations of sodium in the plasma and in the distal tubular urine. After the collection of normal stop-flow urine, the concentration of sodium in plasma was raised rapidly by the intravenous infusion of 2 to 3

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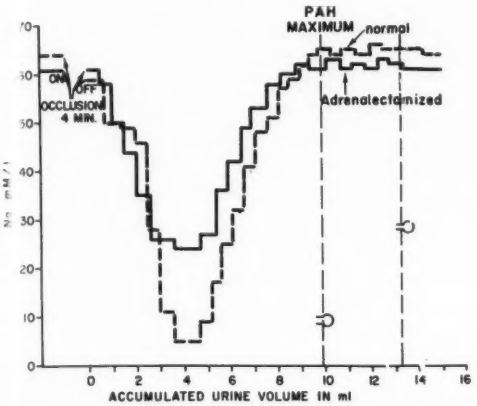

Figure 1

Comparison of the distal tubular concentrations of sodium during stop-flow in a normal and in an adrenalectomized dog. (Republished by permission of the Proceedings of the Society for Experimental Biology and Medicine.[2])

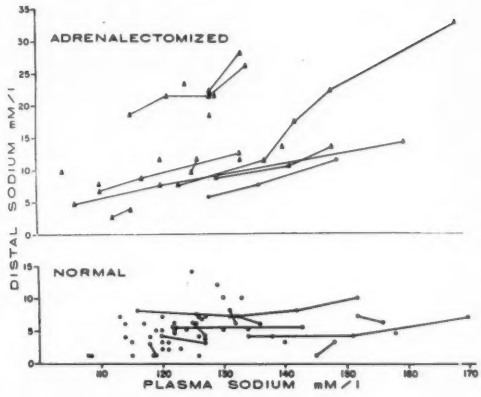

Figure 2

Relationship between the concentrations of sodium in the plasma and in the distal tubular urine during stop-flow. The vertical axis represents the concentration of sodium in the particular urine sample which is acted upon by the maximal number of distal tubules during ureteral occlusion.

Gm. of sodium chloride in 50 ml. of water, over a period of 5 minutes. After 10 more minutes, the occlusion was again performed, and another collection was made.

Figure 2 demonstrates the relationship between the concentration of sodium in the plasma and in the distal tubular urine during ureteral occlusion. It can be seen that during stop-flow, the distal tubule of the normal animal was capable of lowering the concentration of sodium almost to zero. Except possibly at extremely high values, the minimum concentration of sodium appears to be independent of the concentration of sodium in the plasma. This independence suggests that over the physiologic range, the maximal concentration gradient for sodium which the normal animal can develop and maintain between the plasma and the distal tubular urine has not been reached.

The effects of adrenalectomy upon this pattern are striking. Even at very low plasma concentrations of sodium, the distal concentrations during ureteral occlusion were abnormally high. During occlusion, there was a direct relationship between the concentrations of sodium in the plasma and in the distal tubule. This indicates that adrenalectomy has reduced the maximal concentration gradient for sodium which can be maintained across the distal tubular cells. As the concentration of sodium in the plasma rises, the minimal concentration of sodium also rises. Even if the duration of occlusion is prolonged from 4 to 6 minutes, the peak concentration of sodium in the distal tubular urine during stop-flow is not changed, indicating that maximal reabsorption of sodium by the distal tubules occurs within the first 4 minutes of ureteral occlusion.

The effects of aldosterone on the adrenalectomized dogs are demonstrated in figure 3. The administration of aldosterone restored the ability of the distal tubule to lower the concentration of sodium in the distal tubule even in the presence of an elevated concentration of sodium in the plasma.

Mode of Action of Aldosterone

Clearance methods indicate that aldosterone increases the tubular reabsorption of sodium. This action is manifested in stop-flow analysis by the ability of aldosterone to increase the maximal concentration gradient for sodium which can be developed between the plasma and the distal tubular urine. Since the mini-

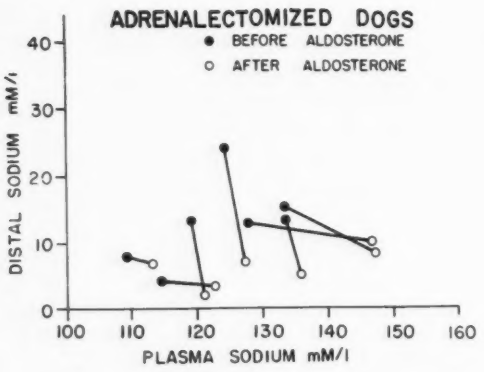

Figure 3

Effect of aldosterone on distal tubular concentration of sodium.

mal concentration of sodium attained in the distal tubule during ureteral occlusion is independent of the duration of occlusion, this concentration must be a steady state value; i.e., the concentration at which sodium movement out of the lumen is equal to sodium movement inward. Aldosterone could act, therefore, in 1 of 2 different ways: it could activate carrier systems responsible for sodium transport outward or it could decrease passive back-diffusion of sodium from interstitial fluid into distal tubular lumen.

A rough estimate of the rate of back-diffusion of sodium has been made in our laboratory, using the isotope Na^{24}. The ureteral occlusion stops filtration so that glomerular substances such as inulin will not enter the concentration pattern except as new filtrate. Na^{24} injected intravenously after the occlusion, by crossing the tubular epithelium transmurally, enters the urinary pattern ahead of inulin. Precise delineation of rates of movement into the stop-flow pattern is complicated by the continuing decline of the precursor Na^{24} in the blood plasma during the short 2-minute period allowed for the Na^{24} to enter the tubule lumen. The shape of the stop-flow curves for Na^{24} seems unchanged after adrenalectomy, with no suggestion of any increased rate of back-diffusion. These preliminary data coupled with the stop-flow data lead us to believe that aldosterone increases the maximal transport velocity by increasing the number of carrier sites available for the reabsorption of sodium.

Potassium

It has been established that potassium is reabsorbed and secreted by the nephron. The area involved in the secretion of potassium is located in a distal region of the mammalian nephron, which is also a site for the secretion of hydrogen.[3] The secretion of ammonia is also thought to take place in the same distal area.[4] Recently, Pitts, Gurd, Kessler and Hierholzer have shown that these secretory areas do exist at the same distal tubular site.[5] On the basis of clearance studies, the secretion of cations is believed to be coupled with sodium reabsorption.[6, 7]

Using the stop-flow technic, we have obtained data which indicate that the secretion of potassium, hydrogen and ammonium occur in a very distal area of the nephron. Figure 4 shows a normal stop-flow curve on which sodium, potassium and hydrogen (titratable acidity) are plotted. The concentration of both potassium and hydrogen rose in the early distal samples and reached maximum values even before the concentration of sodium reached its minimum. Potassium concentrations then fell very sharply to values below those observed during free flow. This drop occurred in the area of low concentrations of sodium. As proximal fluid entered the collector, potassium concentrations returned to free flow values.

We believe that the increase in the concentrations of potassium in the early samples represents secretion of that cation in the collecting ducts and perhaps in some part of the distal tubule. This increase occurs in the first few samples, in urine which must have been trapped in the collecting ducts. This interpretation agrees with the findings of Ullrich et al., who demonstrated that the concentration of potassium may increase in the collecting ducts.[8] Unfortunately, it is impossible for us to delineate more precisely the anatomic areas involved in the secretion of potassium. The secretory area may very well extend into the distal tubule itself.

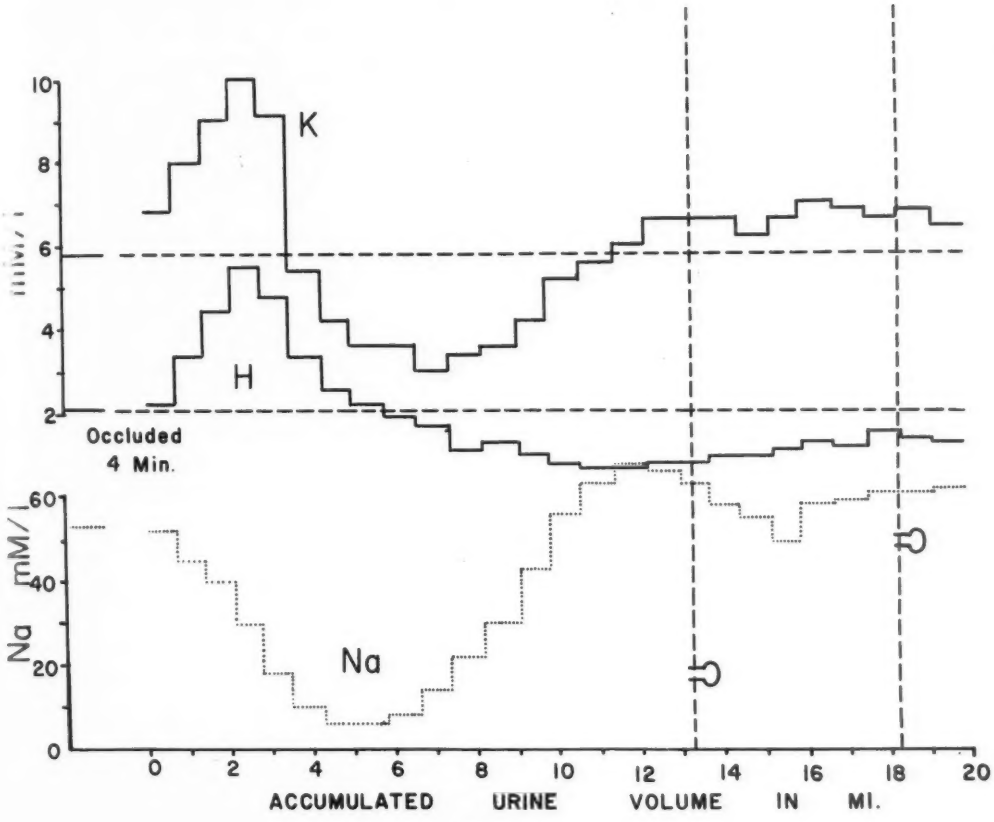

Figure 4

Stop-flow pattern for sodium, hydrogen and potassium.

The minimum concentration of potassium always occurred in a distal area of the stop-flow pattern and was often below the concentration in plasma. We regard this as evidence for the reabsorption of potassium in an area of the distal tubule which also reabsorbs sodium to low levels.

Bott[9] has demonstrated that throughout the proximal tubule of the amphibian nephron, the concentration of potassium remains at the level in the plasma. There is little doubt that the reabsorption of potassium against a chemical gradient does occur. Subjects in potassium deficiency excrete urine with concentrations of potassium lower than that in plasma.[10] Since the reduction in the concentration of potassium in amphibia does not seem to occur in the proximal tubule, it must take place in a more distal area. It is not surprising, then, that the stop-flow patterns for potassium include a distal area of low concentrations of potassium.

The Effects of Impermeant Anions

Infusions of relatively impermeant anions, such as ferrocyanide, sulfate, thiosulfate and phosphate, retard the reabsorption of sodium in the proximal areas of the nephron because of their charge and their inability to accompany reabsorbed sodium across the tubular wall. Thus, it has been suggested that these anions increase the secretion of hydrogen and potassium because they deliver greater amounts of sodium to a distal mechanism

which reabsorbs sodium in exchange for secreted potassium and hydrogen.[6] However, the data derived from the use of these anions in stop-flow analysis have led us to conclude that the effect of these anions upon the secretory mechanism is only part of their total influence upon hydrogen and potassium excretion.

The results of these stop-flow experiments show that the presence of impermeant anions within the tubule not only increased the secretion of hydrogen, ammonium and potassium, but also caused the points of maximum titratable acid and of maximum concentrations of potassium in the stop-flow patterns to be shifted proximally. The maximum concentrations of these substances then appeared in the area of the patterns which normally contained the minimum concentrations of potassium. An area of the nephron, much larger than was apparent in the control occlusions, seemed to be secreting hydrogen and potassium.

Figure 5 shows the results of 1 experiment in which a control occlusion was followed by a second after the infusion of phosphate so that the plasma phosphate level rose from 1.7 mM per liter to 14.3 mM per liter. The control patterns for potassium, ammonium and hydrogen all showed a distal secretory peak which was distal to the sodium minimum. After the infusion of phosphate, the maximum concentrations of potassium, ammonium and hydrogen were all increased. Their maximum concentrations were also all moved more proximally so that they coincided with the minimum for sodium. After infusion of phosphate, the distal reabsorptive area for potassium was no longer evident. Similar results are obtained if the dog is infused with any other relatively impermeant anion, e.g., thiosulfate, ferrocyanide or sulfate.

The stop-flow patterns indicate that, in the presence of the impermeant anions, the apparent secretion of hydrogen and potassium occurred along the full length of the distal nephron in which the strong sodium reabsorptive mechanism is located. Ordinarily, chloride follows the reabsorbed sodium at this site. However, in those animals which are infused with the sodium salt of the impermeant anions, more sodium than chloride is presented to the distal reabsorptive system, and the amount of sodium which is reabsorbed exceeds the supply of chloride. This excess of reabsorbed sodium over chloride cannot be accompanied by the infused anion because of its size and immobility. Thus, as sodium is removed, the anionic charge remaining in the tubule will attract other available cations to replace the sodium. The only relatively diffusible cations available in quantity are hydrogen and potassium, and they are drawn into the tubule from either the tubular cells or the interstitial fluid. Evidently the potassium reabsorptive mechanism cannot counteract this inward movement. In effect, then, a pseudo-exchange mechanism, created by the combination of the reabsorption of sodium and the presence of immobile anions, has been set up in a part of the tubule where one was not apparent in control experiments.

Countercurrent Multiplier System

It has been established that the ability of the kidneys to concentrate urine is dependent upon a countercurrent multiplier system.[11] In this system, the interstitial osmotic pressure increases along the pyramids, being highest at the tip. In the presence of antidiuretic hormone (ADH) this gradient will cause water to be drawn out of the collecting ducts and so concentrate the urine. However, under certain conditions, urine osmolality may decrease even in the presence of maximum ADH. During osmotic diuresis urine osmolality decreases and approaches plasma osmolality as the diuresis becomes more severe.[12]

This inability to concentrate during osmotic diuresis might be considered to result from increased rate of flow through the collecting ducts with concomitant decrease in time allowed for osmotic equilibrium. If this were true, one would expect urine osmolarity to increase significantly during stop-flow as additional time is allowed for equilibration.

Figure 6 shows the results of a stop-flow experiment in which urine osmotic pressure was measured. Although the osmotic pressure

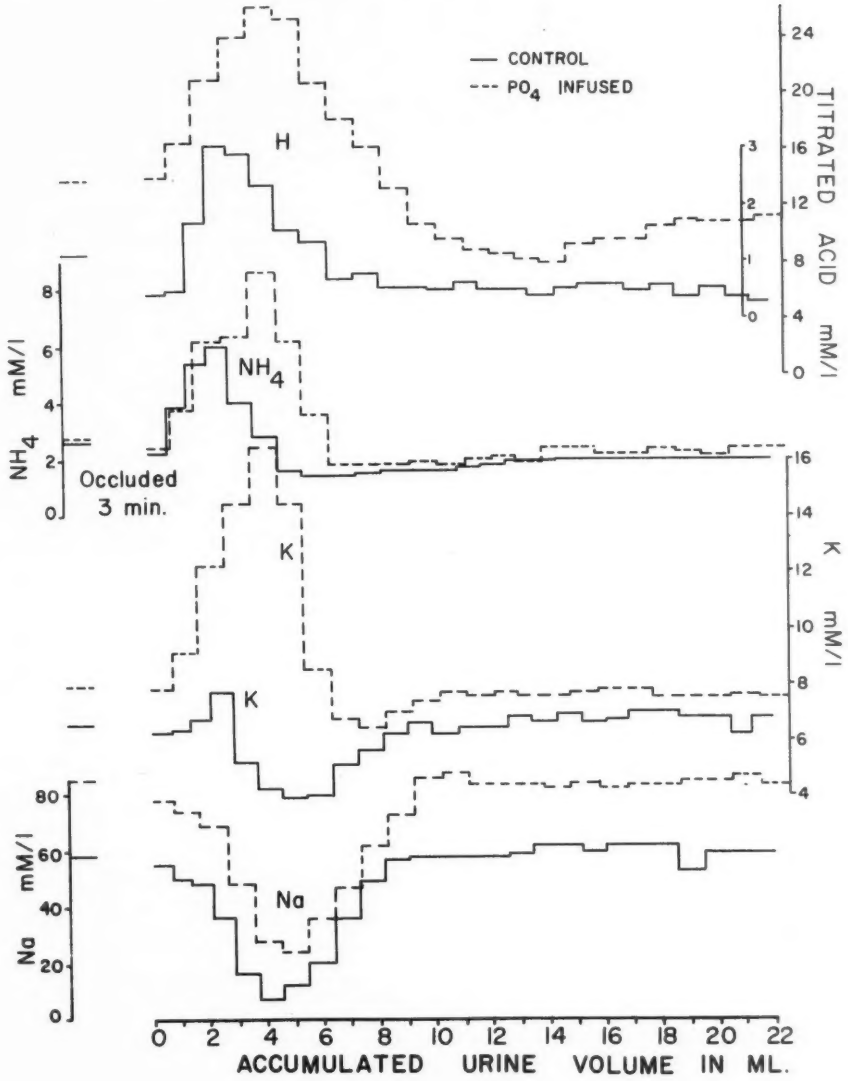

Figure 5

Effect of phosphate on the stop-flow patterns for potassium, ammonium and hydrogen.

did rise in a distal area, the increase was only about 10 per cent of the control osmolarity (from 496 to 542 mOsm. per liter). These results suggest that the osmotic pressure of the interstitium is higher than urine osmolarity during osmotic diuresis, but only slightly so. Since urine osmolarity does increase during stop-flow, this is evidence that during osmotic diuresis the collecting duct urine does not reach osmotic equilibrium with the interstitium.

These results are consistent with the formulations of Hargitay and Kuhn[13] that the rate of urine flow through the loops of Henle is 1 of the determinants of the magnitude of the countercurrent gradient which the kidney

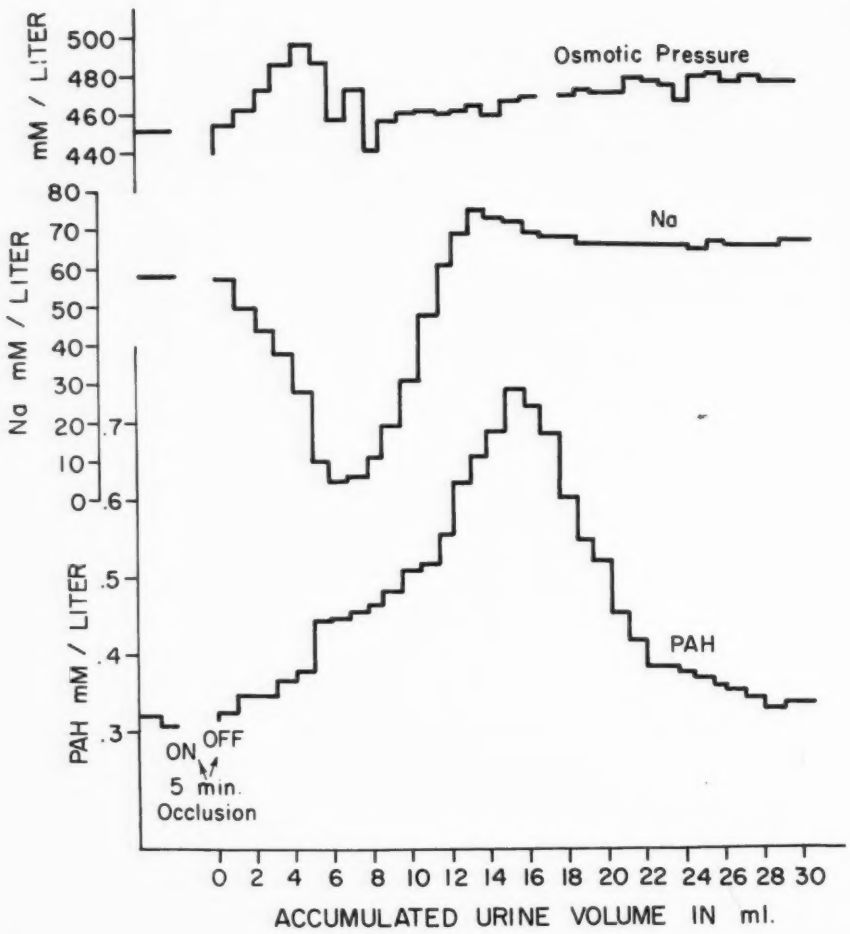

Figure 6

Stop-flow pattern for para-aminohippuric acid (PAH), sodium. (Republished by permission of the American Journal of Physiology.[13])

may establish. There must be an optimum flow rate through the loops. Any increase in flow above that optimum would serve to carry off solute and lessen the osmotic gradient existing in the renal medulla.

Figure 7 shows the results of an experiment which indicates that this is true during osmotic diuresis.[14] Catheters were placed in the left and right ureters and in the left renal artery. After a control period, 20 ml. of 20 per cent mannitol was injected into the left renal artery over a 1-minute period. One-minute urine samples were collected simultaneously from the 2 ureters, and the samples analyzed for their concentrations of sodium. The urine flow from the left kidney increased almost immediately, reaching a maximum during the third minute, then declined relatively slowly. The control sodium concentration in urine from the left kidney was 39 mEq. per liter. As the urine flow increased, so did the concentration of sodium. In the second sample after injection, the concentration of sodium reached 117 mEq. per liter and then declined

t 23 mEq. per liter. A similar pattern was s n on the right side: as the urine flow in- c eased, so did the concentration of sodium. A gain the concentrations of sodium fell off n ore quickly than urine flow. This experiment s ggests that a sudden increase in flow rate t rough the nephron is able to sweep excess s dium out into the urine. This observation is c nsistent with the view that the rate of the f w of urine through the loop of Henle and t e collecting ducts is a determinant of the c untercurrent gradient which may be main- t ined by the kidney.

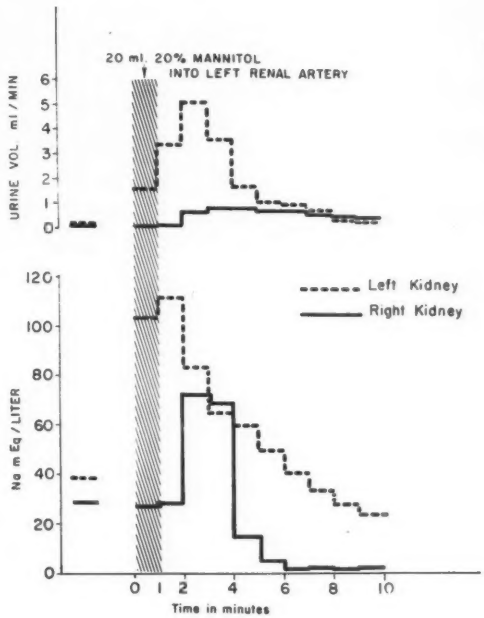

Figure 7

Effect of the injection of mannitol into the renal artery on the flow of urine and the concentration of sodium in the urine.

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Discussion

Dr. Wilde: I should like to consider why, in stop-flow, potassium appears not to be secreted extensively when the supply of sodium is low. My remarks pertain particularly to Dr. Berliner's comment concerning our minimum inflection in the stop-flow pattern for potassium.

I shall assume that at least part of the potassium secretion occurs at the same site as the final reabsorption of sodium. In this way there could be a coupled exchange of sodium and potassium. In accord with Dr. Giebisch's studies on the kidney, and with the studies of others using frog skin, we would expect only a small, negative luminal potential at our low-sodium reabsorptive sites just before the steady state concentration of sodium is achieved.

More important than the low potential is the lack of anions in the lumen to associate with potassium: by the time the final steady state concentration has been reached, the chloride has followed the sodium out of the lumen; early in stop-flow, before all the chloride had followed sodium in reabsorption, chloride is available to associate with any secreted potassium, and the potassium concentration in the lumen would be sustained momentarily. As stop-flow is prolonged, even this potassium chloride might back-diffuse out of the lumen.

This latter suggestion fits our own observations and those of Skulan, Williamson and Shideman at Madison. During stop-flow in rats they found the secretory K peaks to be higher after brief ureteral occlusion than after prolonged occlusion.

In the presence of impermeant anions, potassium concentration forms a high peak at the minimum for sodium. The influence of impermeant anions, such as sodium sulfate, on potassium secretion is that of a nondiffusible counterion for potassium as well as a source of sodium for a sodium-potassium, coupled exchange mechanism.

Chairman Pitts: They seem to be in agreement with you.

Dr. Phyllis Bott: Do I understand from what Dr. Malvin said and also you, Dr. Berliner, that the greatest or the lowest concentration of potassium seems to be in the distal tubule?

Dr. Berliner: You might understand that from what Dr. Malvin said, but I hope not from what I said. There is little doubt that those stop-flow samples which come out of the tubules with the lowest potassium concentration are those which have been in the distal tubule during the period of stop-flow. The question is whether this means that this same pattern was present at the instant at which flow was reinstituted. I would say that we are really not in a position to draw any conclusions about the latter question, although Dr. Malvin disagrees with this view.

Dr. Bott: I want to say, in addition to your comments on the various figures which were put out from our laboratory, that we did (in a preliminary series with chemical methods) get some apparent drops in concentration of potassium in the proximal tubule. I have not found exactly what was wrong with those samples. It may be that they were stored too long. They had to be kept awhile before they were analyzed. That was true both of the Necturus experiments and those on rats that I ran with another one of my "sons," Dr. Wirz. By the way, I am glad to have both of these men (Dr. Giebisch and Dr. Wirz) as sons. I do think that the later results using flame photometer are better. We also published, last spring, some additional results for the rat; here too, the concentration of potassium in proximal tubule fluid does not seem to differ very much from that of serum. However, we have not been able to take fluid from the end of the proximal tubule in the rat.

Question: Please repeat the question.

Chairman Pitts: I would rather repeat what Dr. Bott just said. I am afraid I can't get all the nuances into this, but, in essence, I think what Dr. Bott said is that she does not know why, in some of the early potassium samples collected from the proximal tubule, the potassium was low. Perhaps they stood too long,

or something else. But, for the most part, and especially in more recent work, the concentration of potassium within the proximal tubule has been essentially that of the plasma, that is, equivalent to or equal to the plasma concentration.

The question concerns the potassium dip in the distal tubule. Dr. Malvin, would you like to comment?

Dr. Malvin: Dr. Sullivan has just completed only 1 experiment which has some bearing on the question. He placed a catheter in the renal artery and then infused mannitol in the usual way. Two minutes before clamping the ureter, he infused sodium chloride, in very high concentration and at a very rapid rate, into the renal artery. He continued this infusion during the period of occlusion and during the collection of the stop-flow samples.

Under these conditions, the sodium concentration achieved in the distal tubule was nowhere near as low as that which is usually achieved. In 1 trial, the minimum sodium concentration was a little more than 20 mEq./L. in contrast to the usual concentration of approximately 5. Under these conditions he still obtained a distal dip for potassium, indicating, I think, that even in the presence of adequate quantities of sodium, the potassium concentrations did decline.

Dr. Bartter: I would like to ask Dr. Malvin if he considers that his results have raised the question of an action of aldosterone on the proximal tubule.

Dr. Malvin: About all I can really say is that we were unable to detect any action using the stop-flow method. The only method we have to detect a proximal effect is to look at the creatinine concentration; that is, in fluid coming from the proximal area the creatinine concentrations are higher than in control urine. This could only be due, of course, to water removal, and one can get a fairly good estimate of the volume of water removed during the stop-flow procedure. The volume of water removed is the same in the intact and in the adrenalectomized animal.

Dr. Wakim: I should like to ask Dr. Malvin how long he gave aldosterone before he did the stop-flow. Also, does he believe that the anesthesia and the mechanical effects of the stop-flow had any effect on the aldosterone action? Finally, did he try the experiment that Dr. Berliner did, i.e., eliminate the annoying effect of a dead space in the renal pelvis by filling it with oil?

Dr. Malvin: The last question is perhaps the easiest: the effect of the dead space doesn't seem to be too annoying because the patterns actually are quite similar. With respect to the first question, we injected aldosterone and did the stop-flow between an hour and a half and two and a half hours after the injection of aldosterone.

Dr. Hilton: I should like to ask Dr. Malvin just how physiologic he thinks his technic is. At the levels of intraluminal hydrostatic pressures which he uses, perhaps all of the effects on sodium and water can be explained by just mechanical diffusion, i.e., by the pushing of sodium and water out of tubule across stretched membranes. In this way, the potassium, PAH and creatinine, would all be concentrated.

Dr. Malvin: No, because, for one thing, many solutes do not concentrate. Secondly, of course, I am not sure that I know of any experiment which is physiological.

Dr. Hilton: This is abnormal kidney, definitely?

Dr. Malvin: Of course.

Dr. Hilton: You see this thing in prostatic obstruction.

Dr. Searle B. Rees: I wonder if Dr. Giebisch observed any bidirectional flux of potassium? His very pretty model did show potassium diffusion downhill.

Dr. Giebisch: No, I did not observe any.

Dr. Wilde: If I may say something more about the oil drop or the injection of oil into the pelvis. Although this method truly is a help in collecting the *first* samples and getting a better idea of the very first, conduit-type of material that is coming out, it is not an advantage in terms of handling proximal samples.

Dr. Smith: A question has come up as to the effect of the hydrostatic pressure on the

tubules. I don't think the concentration terms here are of the order of magnitude that could be affected by a change in hydrostatic pressure, though by that I would not mean to dismiss the changes in intraluminal hydrostatic pressure as having no significance. But I would like to go back to the philosophy of the experiment.

Dr. Malvin, may I ask you why you put the clamp up on the ureter in the first instance? What did you think would happen?

Dr. Malvin: You mean——?

Dr. Smith: Why did you do the experiment?

Dr. Malvin: Why did I do it?

Dr. Smith: Yes.

Dr. Malvin: I'll have to think. I did it because I thought it would work, I imagine.

Dr. Smith: Well, what did you think would happen to the composition of the urine when you put the clamp on the ureter?

Dr. Malvin: You mean, would the clamp itself affect the urine?

Dr. Smith: No, it's not that tricky. Weren't you, perhaps, laboring under the impression that these transport processes involved some significant factor of time, that they weren't all instantaneously proceeding to the right?

Dr. Malvin: Oh, yes.

Dr. Smith: Have you any evidence at the present time that they are not instantaneous in proceeding to the right?

Dr. Malvin: Surely.

Dr. Smith: You have evidence that equilibrium with respect to potassium, sodium, ammonium, hydrogen ion is not instantaneously reached between the tubular cell and the lumen?

Dr. Malvin: Yes. It seemed to me that if they were, concentrations could not change with respect to time. Do we agree on that?

Dr. Smith: Well, let me ask another question by way of defending the first. What happens if, immediately after you put the clamp on the ureter, you inject inulin intravenously and wait for 4 to 6 minutes and take the clamp off?

Dr. Malvin: If you inject inulin after the clamp is put on ——

Dr. Smith: Immediately.

Dr. Malvin: Unfortunately, we have never done this immediately after the clamp. I think the shortest time interval was about 4 minutes. Then, you find inulin enters the tubular fluid downstream from where one would expect new filtrate to come in. For example, if you clamp, 4 minutes later inject inulin, 2 minutes later creatinine, the inulin will appear before the creatinine, and so on. However, if you wait, let's say 8 or 10 minutes, perhaps 10 minutes, and then do this same routine, they appear together. But it looks as though this water movement down the tubule does go on relatively rapidly at first and then tapers off, and perhaps stops; I don't know.

Dr. Smith: Well, not perhaps. There are others up there who have used the stop-flow technic. Dr. Berliner, or Dr. Pitts, have you ever injected inulin immediately after the clamp and then released it after 4 minutes?

Chairman Pitts: Yes, and it appears in the first sample.

Dr. Smith: Which brings me back to my question. Why did you put the clamp on in the first instance?

Dr. Malvin: What would you like me to say? (Laughter)

Dr. Smith: I'm wondering whether, Dr. Malvin, with the stop-flow technic, you have really dismissed the possibility that the reactions between the epithelium and the urine in the lumen attain equilibrium instantaneously, and if the continued movement of fluid down the nephron is not somehow, in some instances, giving you something of a smeared picture.

Dr. Malvin: I'm sure there is smearing. However, one can still exclude instantaneous equilibrium. For example, glucose is reabsorbed from proximal samples and declines in concentration in the stop-flow pattern. Now, it's hard to see how you could have declining glucose concentrations when, if anything, higher concentrations are coming in from the plasma.

Dr. Smith: I'll rest.

Dr. Wilde: We first did the stop-flow experiments to try to elucidate some of the factors involved in osmotic diuresis with mannitol; indeed, that was the immediate source

of the invention. Now, with respect to osmotic diuresis during free flow, why does sodium not fall any lower than it does? Does the high value for the minimum sodium concentration during free flow represent a consequence of time limitations or is it a true steady state value, indicating a feeble transport system? The low concentrations seen at the distal minimum during stop-flow indicate that during free flow a steady state is not attained at this site.

Dr. Smith: May I have one more comment?

Chairman Pitts: Could we have one from Dr. Mauro?

Dr. Mauro: I would like to ask Dr. Giebisch to tell us a little bit about the sensitivity of the transmembrane potentials to anoxia and to various other types of metabolic poisons. Is this picture radically different from what is seen in the frog skin or in the various bladder preparations?

Dr. Giebisch: As far as ischemia or metabolic poisons are concerned, there seem to be no basic differences. Particularly under conditions of ischemia, renal cellular potentials behave quite similar to other structures such as nerve or muscle: a rapid phase of partial depolarization is followed by stabilization of the transmembrane potential at about 60 to 70 per cent of the initial potential difference.

Regarding metabolic poisons, my own experience is limited to organic mercurials. There, a significant depolarization occurs after the administration of Neohydrin. We had the good fortune to be able to use mercury^{203}-labeled Neohydrin and accordingly could measure the degree of depolarization as a function of the actual concentration in the renal tissue. A fairly good relationship obtains so that in the Necturus kidney increasing depolarization can be observed with higher amounts of Neohydrin. It might also be mentioned that ischemia and organic mercurials have additive effects in depolarizing single proximal tubule cells.

Other relevant observations concerning transtubular potential differences are those of Whittembury and of Schatzman, Windhager and Solomon. These authors found that 2,4-dinitrophenol (2×10^{-4}M) reduces the transtubular potential difference by about 50 per cent. To my knowledge, no other metabolic inhibitors have been tested so far.

Dr. Strauss: I would like to ask Dr. Malvin whether he was suggesting that aldosterone had a permissive action on sodium reabsorption?

Dr. Malvin: There is very little I can say about the actual mechanism of sodium reabsorption, particularly how aldosterone affects it. I can only give you the data and suggest that it does affect the transport system for sodium. Precisely how, I don't know.

Dr. Smith: Mr. Chairman, may I ask Dr. Strauss a question? Given the premise that putting the clamp on the ureter prolongs the exposure of the urine to the various segments of the tubule, then increasing exposure time is equivalent to reducing the velocity of flow down the tubule. We have that experiment in man, in oliguria, and in water diuresis; assuming the same conditions of sodium balance, I wonder if Dr. Strauss can quote, offhand, the minimal sodium concentration in oliguric urine as compared with water diuresis where flow is free?

Dr. Strauss: I am not sure that I can, but I have the impression that it tends to be just as low in either situation. I am not sure.

Chairman Pitts: Isn't it true that A. V. Wolff found a minimum of sodium concentration—I guess it was chloride, not sodium—in sustained high water diuresis, something of the order of 30 or 33 mEq. per liter of urine, I believe?

Dr. Smith: That is a slightly different situation. I am thinking about a man. Under presumably the same conditions of sodium balance, does sodium concentration tend to be lower during water diuresis when the flow is large, the velocity is great and the time exposure is small, than during oliguria, when the velocity is low? So I wonder to what extent the time of exposure really does play a part in, let's say, sodium reabsorption in the collecting duct system.

Dr. Malvin: The only information I have

bearing on this—unfortunately, I can't cite the precise figures—is that if you occlude, let's say, for a minute and a half, the minimum sodium is not as low as if you occlude, say, for 4 minutes. So that the value does decline with time.

Dr. Ullrich: May I ask a theoretical question of Dr. Giebisch? If you assume that water is moved out of the tubule by a force and that the solutes diffuse out, then a perfusion potential may originate. How high do you think this could be?

Dr. Giebisch: Are you postulating a system where you get intratubular negativity by moving water out by bulk? Such a system would require not only a driving force to get the water out but also a special device for leaving chloride behind. I think that the special device must also exist before you can postulate bulk flow as the means of creating a potential difference. I think that if you postulate a perm-selective membrane, and move water out and leave an anion behind, you can get potential distribution which is similar to any naturally observed one.

Dr. Ullrich: I ask this because Dr. Eigler and I have measured intracellular potentials. We used the same solute to perfuse the renal tubules inside and outside so that we could have the same electrochemical potential inside and outside. Then we measured the transtubular potential of the frog tubule. It was about 10 millivolts; negative inside and positive outside. Then, if we perfused the inside with indigo carmine solution, we measured an increase of negativity on the inside of the order of 20 millivolts, i.e., from 10 to 30. What do you think about that? Is it a diffusion potential, because indigo carmine is an acid and the H^+ ion will diffuse out more quickly than the large indigo carmine ion?

Dr. Giebisch: I think that you have suggested a likely explanation, but I have no actual basis for comment since I have never used indigo carmine.

Dr. Ullrich: On the other hand, in in vivo preparations of the guinea pig and the frog tubules, we found an intracellular potential of the same magnitude as you found in Necturus: in the proximal tubule of the guinea pig about 70 to 80 millivolts, and in frog about 70 millivolts. In vitro the intracellular potential was much lower; in the proximal tubule about 60, in the cells of outer medullary region about 50, and in the cells of inner medullary region about 40 millivolts.

"Water Is Best"

It was assuredly not chance that led Thales to found philosophy and science with the assertion that water is the origin of all things. Whether his belief was most influenced by the wetness of animal tissues and fluids, or by early poetic cosmogonies, or by the ever present importance of the sea to the Ionians, however vague his conception of water may, indeed must, have been, he at least expressed a conclusion which proceeded from experience and serious reflection.—L. J. Henderson. *The Fitness of the Environment.* Boston, Beacon Press, 1958, p. 72.

III. LESSONS FROM COMPARATIVE BIOLOGY

Chairman: Alfred P. Fishman, M.D.

INTRODUCTION

By ALFRED P. FISHMAN, M.D.

Every generation of scientists includes a few who are not only able to detect in their own special research a general pattern of nature, but are also endowed with an extraordinary capacity for communicating their ideas. This combination of conceptualization and communication has led to such great generalizations in biology as "natural selection," "the constancy of the internal environment" and "the fitness of the environment."

Our speaker for tonight, Professor George Wald, is one of these gifted men. His special interest in biology is the chemistry and physiology of vision—in particular, of the visual pigments of the rods and cones of the retina. This special interest has led him, by the path of biochemical evolution, to vertebrate metamorphosis, and from there, to an appraisal of light and life, and of the origin of life. The particular lesson which he brings to this symposium is the use of comparative biology to expand specialized biological knowledge into general biological concepts.

George Wald is Professor of Biology at Harvard College. Over the years, his scientific accomplishments have been recognized in many ways; the most recent honor being the Rumford Premium of the American Academy of Arts and Sciences. It gives me great pleasure to introduce to you Professor Wald.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, N. Y.

The Significance of Vertebrate Metamorphosis

By GEORGE WALD, PH.D.

It is generally recognized that the anatomical metamorphosis of the vertebrates seems to recapitulate their evolutionary history. However, little attention has been paid to the biochemical changes which precede or accompany the anatomical changes. In the present paper, the author uses the biochemistry of vision as the point of departure for considering the parallel changes in biochemistry, anatomy and ecology which are involved in vertebrate metamorphosis.

"Nicodemus saith unto him, How can a man be born when he is old? can he enter the second time into his mother's womb and be born?

"Jesus answered, Verily, verily, I say unto thee, Except a man be born of water . . ."—John, 3:4, 5.

ONE OF Thomas Mann's novels begins with the words, "Very deep is the well of the past." This is the well from which all biology is drawn. Physics and chemistry deal with what is; biology with what has become. In biology one is never far from history; and each living organism carries fragments of this history with it, as vestiges and recapitulations, anatomical and, as we shall see, biochemical.

I was led into this subject through the study of visual systems. I shall begin with them since they still provide the main thread of the argument, though by now it branches widely.

What we are concerned with here is an aspect of biology at the molecular level. Like all such developments, it runs closely parallel with earlier arguments based primarily upon anatomy. This is hardly surprising, for the anatomy of an organism is the greatly magnified expression of its chemistry; and the short- and long-term changes in anatomy that constitute the organism's embryogeny and evolution, by the same token, express chemical changes. The biochemist is a biologist whose dissections have reached the molecular level. Far from removing him from biology, this gives him new opportunities to pursue it.

For like reasons, some of the viewpoints and conclusions expressed here were reached long ago by other paths. They will bear this reiteration and the new support that biochemistry brings them.

From the Biological Laboratories, Harvard University, Cambridge, Mass.

This paper is a revised version—in a sense a second edition—of one that appeared earlier in Science.[1] The material from the earlier version is republished by permission of Science. The investigations from our own laboratory were supported in part with funds from the Rockefeller Foundation and the Office of Naval Research.

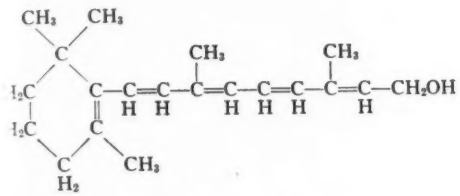
Primacy of the Spawning Environment

Two kinds of visual system are found in the rods of vertebrate retinas. One is based upon the red visual pigment, rhodopsin, formed by the combination of the protein opsin with retinene, the aldehyde of vitamin A. The other is based upon the purple pigment, porphyropsin, formed from the same type of opsin combined with retinene$_2$, the aldehyde of vitamin A$_2$. Retinene$_2$ and vitamin A$_2$ differ from retinene and vitamin A only in possessing an added double bond in the ring (fig. 1).[2-5]

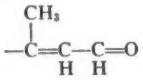
The porphyropsin system was first discovered in fresh-water fishes. Marine fishes and land vertebrates characteristically possess the rhodopsin system (figs. 2 and 3).[6, 7]

What of the fishes that are neither fresh-water nor marine, but migrate between both environments? It would be well before discussing them to clarify somewhat their biological position.

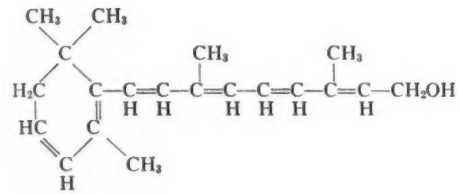
Most fishes are restricted throughout their lives to narrow ranges of salinity. Such forms are called "stenohaline" and are of 2 kinds, fresh-water and marine. A much smaller group of fishes can live as adults in a wide



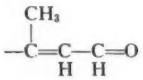
Vitamin A, $C_{20}H_{29}OH$



Retinene, $C_{20}H_{28}O$



Vitamin A_2, $C_{20}H_{27}OH$



Retinene$_2$, $C_{20}H_{26}O$

Figure 1

Structures of vitamin A, retinene, vitamin A_2, and retinene$_2$.

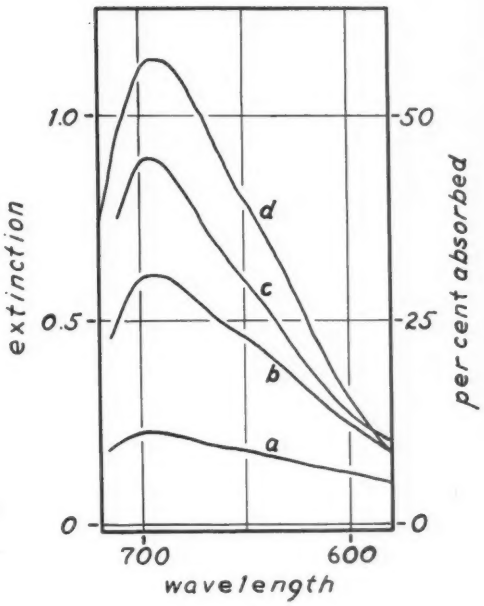


Figure 2

The vitamin A_2 of the retinas of fresh-water fishes. Antimony chloride tests with extracts of wholly bleached retinas display only the absorption band maximal at 690 to 696 mμ characteristic of vitamin A_2. This result has been obtained invariably in about 12 widely distributed species of fresh-water teleost. In almost all cases the visual pigment has also been extracted and this pigment has been found to be porphyropsin, with λ_{max} about 522 mμ. (Republished by permission of the Journal of General Physiology.[6])

range of salinities. They are called "euryhaline," and, again, are of 2 kinds, anadromous and catadromous, meaning "upstream" and "downstream." These terms refer to the direction of the spawning migrations. Salmon, for example, are typically anadromous forms, coming upstream to spawn, whereas the "fresh-water" eels are catadromous, going downstream to the sea on their spawning migration.

It is probably true, however, that no euryhaline fish has to leave its spawing environment to complete a normal life cycle. Many instances are known in which anadromous fishes remain permanently in fresh water. The same is true of such an anadromous cyclostome as the sea lamprey, which has recently colonized the Great Lakes and virtually destroyed the fresh-water fisheries there.

So far as we know, the spawning environment is always fixed. The eggs, the sperms, or the embryos, perhaps sometimes all 3, are stenohaline. Euryhalinity develops later in life and permits, though does not compel, these animals to migrate to the other environment. Migration is only a potentiality, which some of these forms exploit regularly and others rarely. The salmons are essentially fresh-water fishes with the privilege of going to sea as adults; the fresh-water eels are marine fishes with the capacity of coming as adults into fresh water.

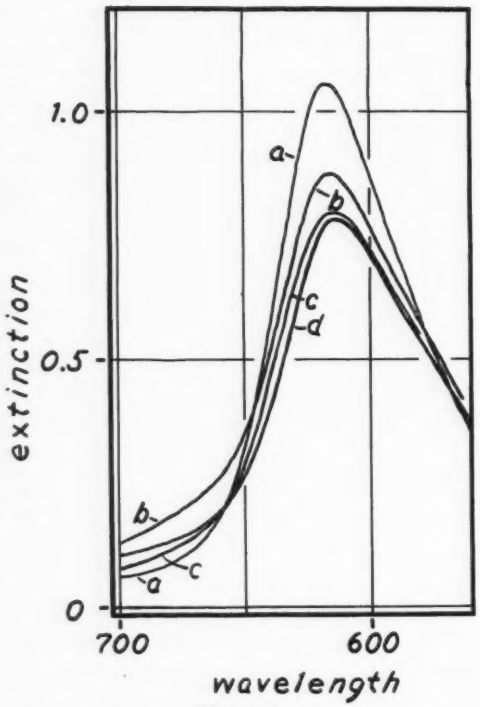


Figure 3

The vitamin A_1 of the retinas of marine fishes. Spectra of the antimony chloride tests with extracts of bleached retinas reveal the λ_{max} at 615 to 620 mμ characteristic of vitamin A_1. This result has been obtained with a great variety of bony, and a few elasmobranch, fishes and is characteristic also of land vertebrates. Two wrasse fishes (Labridae), however, the cunner and tautog, though wholly marine, are exceptional in having a predominance of vitamin A_2 in their retinas. (Republished by permission of the Journal of General Physiology.[6])

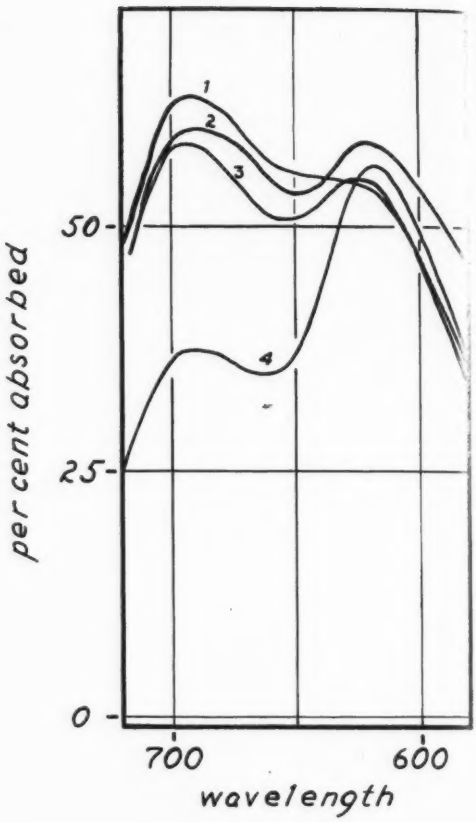


Figure 4

Retinal vitamins A in euryhaline fishes. Spectra of antimony chloride tests with extracts of bleached retinas from (1) chinook salmon, (2) rainbow trout, (3) brook trout, and (4) the American "fresh-water" eel. All these tissues contain both vitamins A_1 and A_2, the anadromous salmonids a predominance of vitamin A_2, the catadromous eel a higher proportion of vitamin A_1. (Republished by permission of the Journal of General Physiology.[6])

The significant biological statement concerning such fishes is not that they migrate but that, being fixed in spawning environment, they are euryhaline as adults. I should like on this basis to redefine the terms applied to them. An anadromous fish is a euryhaline form which spawns in fresh water; a catadromous fish, one which spawns in the sea.[6]

On examining the visual systems of several genera of salmonids, I found that all of them possess mixtures of the rhodopsin and porphyropsin systems, yet primarily the latter, characteristic of the spawning environment. Conversely, the American fresh-water eel possesses a mixture of both visual pigments, in which rhodopsin—again the spawning type—predominates (fig. 4).[7] Certain other anadromous fishes—alewife, white perch—possess porphyropsin almost alone (fig. 5). All the euryhaline fishes examined follow a simple rule: all of them possess, either predominantly

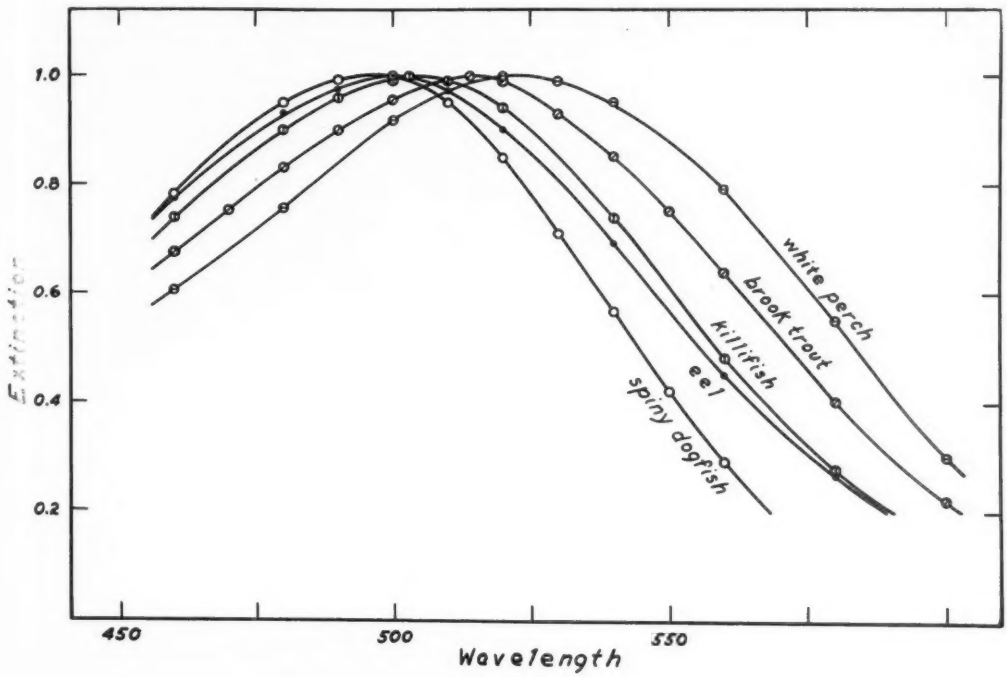


Figure 5

Spectra of the visual pigments of various fishes, illustrating the transition from an exclusively rhodopsin to an exclusively porphyropsin system, correlated with salinity relations. The permanently marine dogfish possesses rhodopsin alone (λ_{max} about 497 mμ); the catadromous eel and brackish-water killifish, predominantly rhodopsin; the anadromous brook trout, predominantly porphyropsin; and the anadromous white perch, wholly porphyropsin (λ_{max} about 522 mμ). (Republished by permission of the Journal of General Physiology.[8])

or exclusively, the type of visual system characteristically associated with the spawning environment.[6, 8]

To a first approximation these patterns are genetic and independent of the immediate environment. The salmonids which were found to possess mixtures of both visual systems had spent their entire lives in fresh water. Alewives just in from the sea on their spawning migration possess porphyropsin almost exclusively. Most striking of all, the cunner and tautog, members of the wholly marine family of Labridae, the wrasse fishes, possess porphyropsin; this is the only type of marine fish yet known to do so.[6, 8]

Since the distribution of visual systems among fishes is genetic, one may ask whether it fits into some evolutionary pattern. Many paleontologists are convinced that the vertebrate stock originated in fresh water. It is from such fresh-water ancestors that our fresh-water fishes were ultimately derived.* The observation that these animals character-

*The "ultimately" here conceals a thorny problem. Fish evolution has probably involved numerous interchanges between fresh-water and marine existence, and many present-day fresh-water fishes may have had marine forms in their ancestry. In that case one should have to assume that the complex of genetic changes that has brought stocks into fresh water has regularly carried with it the property of using vitamin A_2 and porphyropsin in vision. I cannot suggest a genetic mechanism for this association; the association itself is a fact to which as yet no exceptions are known.

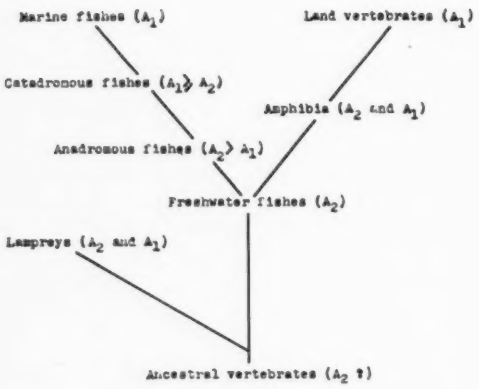


Figure 6

Distribution of vitamins A_1 and A_2 in vertebrate retinas. The observations, all made on contemporary animals, are here correlated with the present ecology. They may also, however, represent evolutionary sequences, and in that case they convey the suggestion that primitive vertebrate vision was based upon vitamin A_2.

istically have the porphyropsin system suggests that this may have been the ancestral vertebrate type—a view that receives some support from the observation that the sea lamprey, *Petromyzon marinus,* a member of the most primitive living group of vertebrates, possesses porphyropsin as an adult[9] (see below). Subsequently vertebrates undertook 2 great evolutionary migrations, one into the sea, the other to land. Both led them to the use of rhodopsin in rod vision, for this is the pigment we find characteristically in marine fishes and land vertebrates. The euryhaline fishes are intermediate between fresh-water and marine forms both in life history and in the composition of their visual systems. In this regard one can arrange the fishes in such an ordered sequence as that shown in figure 6.

Euryhaline Fishes and Amphibia

One can hardly develop such an argument as outlined above without raising questions regarding the amphibia. These animals come between fresh-water fishes and land vertebrates, just as the euryhaline fishes do between fresh-water and marine fishes. Most amphibia, like most euryhaline fishes, spawn in fresh water. Indeed, the life cycle of the common frog runs strikingly parallel with that of such an anadromous fish as the salmon. Both originate and go through a larval period in fresh water. Both, after undergoing deep-seated anatomical and physiological changes which can in both be described as metamorphosis, migrate for the growth phase, the salmon to sea, the frog to land. Both return to fresh water at sexual maturity to spawn. What land is to the frog, the sea is to the salmon. *The euryhaline fishes are the amphibia among the fishes.*

In this sense one might speak of almost all amphibia as "anadromous," meaning that they spawn in fresh water and are free, as adults, to go back and forth between fresh water and the land. [One would like to commit further etymological atrocities. The essence of the euryhaline condition is the capacity to migrate, not so much between low and high salt concentrations as between hydrating (fresh-water) and dehydrating (sea and land) conditions. In this sense the amphibia are "euryhaline." Obviously one needs new terms, firmly grounded in ecological essentials rather than in trivialities.] A few amphibia (red-backed and tree salamanders) have developed special devices for living permanently ashore. I know of no "catadromous" amphibian—that is, one that spawns on land and goes through its growth phase in the water. A number of aquatic reptiles, however (alligators, fresh-water snakes, and turtles), fulfill this description nicely.

If these are substantial parallels, and if the spawning environment decides the pattern of visual pigments, then one should expect such an "anadromous" amphibian as the common frog to possess mainly porphyropsin, like a salmon. Yet rhodopsin was originally discovered in the rods of frogs, and for a long period all that we knew of this pigment was learned with frogs.

In this dilemma I turned to a tailed amphibian with the thought that it might display more primitive properties than the tailless types. Adults of the common New En-

land spotted newt, *Diemyctylus* (formerly *Triturus*) *viridescens*, were found to possess porphyropsin exclusively.[10] This brought a first amphibian into the same fold with certain anadromous fishes but left the frog in a more aberrant position than ever.

On examining bullfrogs in metamorphosis, however, I found that tadpoles just entering the metamorphic climax possess porphyropsin almost entirely, whereas newly emerged frogs have changed almost entirely to rhodopsin.[11] The anatomical metamorphosis, which in this species takes about 3 weeks, is accompanied by this biochemical metamorphosis of visual systems. The bullfrog enters metamorphosis with porphyropsin, like a fresh-water fish, and emerges with rhodopsin, like a land vertebrate (fig. 7).

These observations have recently been confirmed;[12, 13] and Wilt[12] has shown that the metamorphosis of visual pigments in the bullfrog can be stimulated to occur prematurely by treatment with thyroxine. A similar metamorphosis has also been observed in the Pacific tree frog, *Hyla regilla*.[13] On the other hand, several instances have been recorded in which the visual pigment does *not* appear to change at anatomical metamorphosis: the frogs, *Rana esculenta* and *temporaria*,[14] and the toad, *Bufo boreas halophilus*.[13]

Certain amphibia, therefore, like euryhaline fishes, may display both the rhodopsin and porphyropsin systems. It seemed for a time that one difference between both groups might be that in euryhaline fishes the patterns of visual system are fixed, whereas in amphibia they change abruptly with metamorphosis. I shall have more to say of this later.

Biochemistry of Metamorphosis

At the time the metamorphosis of visual systems was discovered in the bullfrog, another such change in the same species had already been described. McCutcheon[15] had found that the properties of hemoglobin in this animal change markedly at metamorphosis. The oxygen equilibrium curve of hemoglobin, measured at one temperature and pH, goes through a remarkable transition between tadpoles and adults. The hemoglobin of tadpoles has a high affinity for oxygen, and it seemed from McCutcheon's measurements that the shape of its oxygen equilibrium curve might be hyperbolic, whereas the hemoglobin of young adults has a relatively low affinity for oxygen, and its equilibrium curve is distinctly S-shaped.

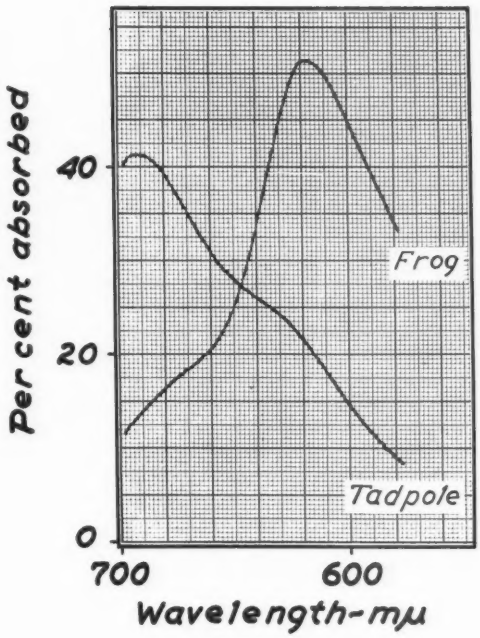


Figure 7

Biochemical metamorphosis of visual systems in the bullfrog, Rana catesbiana. The tadpole just entering the metamorphic climax has in its retina vitamin A_2 (that is, porphyropsin) with only a trace of vitamin A_1 and rhodopsin, whereas the newly emerged froglet has just the reverse pattern. (Republished by permission of the Harvey Lectures.[11])

Riggs[16] re-examined this situation in our laboratory. He confirmed McCutcheon's finding of a striking loss of oxygen affinity at metamorphosis. He found, however, that the shape of the oxygen equilibrium curve does not alter at metamorphosis; it is equally sigmoid throughout development. He found another important change: tadpole hemoglobin exhibits almost no loss of oxygen

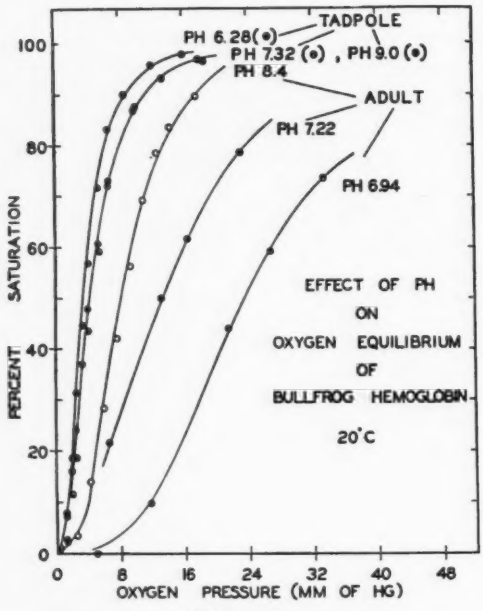


Figure 8

Biochemical metamorphosis of hemoglobin in the bullfrog, Rana catesbiana. Tadpole hemoglobin has a high affinity for oxygen and virtually no loss of affinity on acidification (the Bohr effect); adult hemoglobin has a much lower affinity for oxygen and a large Bohr effect. (Republished by permission of the Journal of General Physiology.[16])

affinity on acidification (that is, no Bohr effect), whereas frog hemoglobin has a very large Bohr effect (fig. 8).

It is clear therefore that hemoglobin, like the pigment of rod vision, metamorphoses in the bullfrog at the time of anatomical metamorphosis. Both substances are conjugated proteins. In the rod pigment, it is the prosthetic group, retinene, which changes; the protein opsin, so far as known, remains unaltered. In hemoglobin it is the protein, globin, which changes; the prosthetic group, heme, is the same always.*

Recently Frieden et al.[20] have described a third change in the proteins of this species at metamorphosis (fig. 9). In the bullfrog tadpole the predominant proteins of the blood plasma are globulins. At metamorphosis, the protein concentration of the plasma doubles and albumins become predominant. These changes can be induced prematurely, just as can anatomical metamorphosis, by administering triiodothyronine.

Still another type of biochemical change has been shown to accompany metamorphosis in frogs and salamanders. Fishes excrete most of their nitrogen as ammonia, whereas land vertebrates excrete their nitrogen primarily as urea or uric acid. Munro[21] showed some years ago that whereas the tadpoles of the frog, *Rana temporaria*, excrete the great bulk of their nitrogen as ammonia, at the metamorphic climax this animal goes over to excreting its nitrogen primarily as urea. At this time, also, arginase, the last in the chain of enzymes that forms urea, makes its first appearance in the liver (fig. 10).* Recently Munro[23] has demonstrated similar changes accompanying metamorphosis in the toad *Bufo bufo*, the salamanders *Triturus vulgaris* and *T. cristatus*, and the axolotl *Siredon mexicanum*.

These observations make a beginning with the biochemistry of metamorphosis. They show that just as animals in metamorphosis undergo radical alterations in anatomy, so their biochemistry is fundamentally revised at the same time. Indeed both kinds of change,

*Such changes in hemoglobin seem to represent a fundamental property of vertebrates, for they penetrate to the most primitive forms. Adinolfi, Chieffi and Siniscalco[17] have recently repotted that larvae of the lamprey, *Petromyzon planeri*, possess 2 hemoglobins, and on metamorphosis change to 2 others. Sometimes they found all 4 hemoglobins together in metamorphosing animals. Manwell[18] has reported finding different hemoglobins in postlarval and adult California sculpins (*Scorpaenichthys marmoratus*, a marine teleost). He states also that preliminary experiments on a live-bearing surf perch (*Embiotica lateralis*) reveal a fetal form of hemoglobin. Evidence is beginning to emerge also that certain euryhaline fishes have mixed hemoglobin patterns as adults that parallel their possession of mixed visual systems; for 2 to 3 hemoglobins have been found in each of a variety of salmons, trout and shad; whereas a number of permanently marine and fresh-water fishes each possessed a single hemoglobin.[19a, b]

*Brown and Cohen[22] have recently analyzed in detail the appearance at the time of metamorphosis in the bullfrog, *Rana catesbiana*, of the enzyme system concerned with urea synthesis.

natomical and biochemical, herald an ecologi- l transition, for they are followed by radical anges of habitat. They mark also an evolu- onary transition, for these changes offer the ost striking instances we know of recapitu- tion. The amphibian in metamorphosis ems to repeat in rapid summary the changes hich accompanied the emergence of verte- rates from fresh water onto land. The trans- rmations of visual systems and of the pat- rns of nitrogen excretion seem to provide ear instances of *biochemical recapitulation.* he changes in hemoglobin also seem to in- olve aspects of recapitulation.[10, 24] Whether he changes in serum proteins have this char- cter, it is too early to say. In any case, in metamorphosis the anatomy, the biochemistry, and, shortly afterward, the ecology all are transformed, and frequently in some degree of accord with the animal's evolutionary history.

It is interesting to realize how closely these patterns hold together. An aberration in one of them seems to call forth appropriate aberrations in the others. The mud puppy, *Necturus maculosus,* for example, remains to some degree a permanent larva, never losing its external gills and never emerging from the water. Some years ago I found that adult mud puppies have porphyropsin alone, like a freshwater fish.[10]

The clawed toad, *Xenopus laevis,* a member of the peculiar family Aglossa, which possesses neither tongue nor teeth, is a purely aquatic form, which, though it metamorphoses, ordinarily never emerges from the water. Adults of this species possess in their retinas vitamin A_2 and porphyropsin almost exclusively.[25, 26] Underhay and Baldwin[27] have recently shown that this species also exhibits peculiar changes in the pattern of its nitrogen excretion. As a tadpole it excretes nitrogen primarily as ammonia. At metamorphosis, like other amphibia, it begins to change over toward urea excretion, so that at the height of metamorphosis it excretes a little more nitrogen as urea than as ammonia. Toward the end of metamorphosis, however, it swings back again, so that the adult excretes

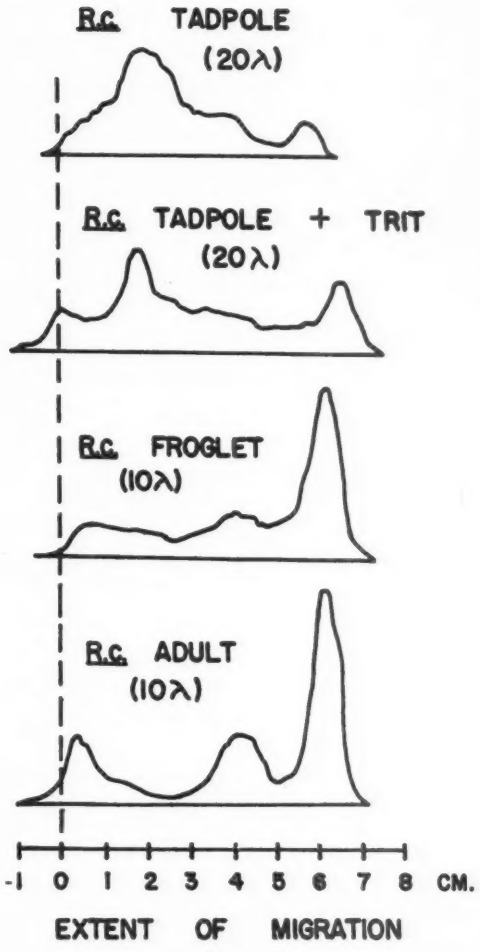


Figure 9

Biochemical metamorphosis of plasma proteins in the blood of the bullfrog, Rana catesbiana. Tadpole plasma contains a predominance of globulins that migrate slowly on electrophoresis at pH 8.6; in the froglet a change to a predominance of rapidly migrating albumin takes place. This change is also induced prematurely by treatment of tadpoles with triiodothyronine (Trit). *The volume of serum used is indicated in parentheses. (Republished by permission of Science.[20])*

about 3 times as much ammonia as urea nitrogen. It is as though this animal, having got ready to leave the water, changed its mind; and both the getting ready and the change of mind are reflected in the nitrogen excretion.

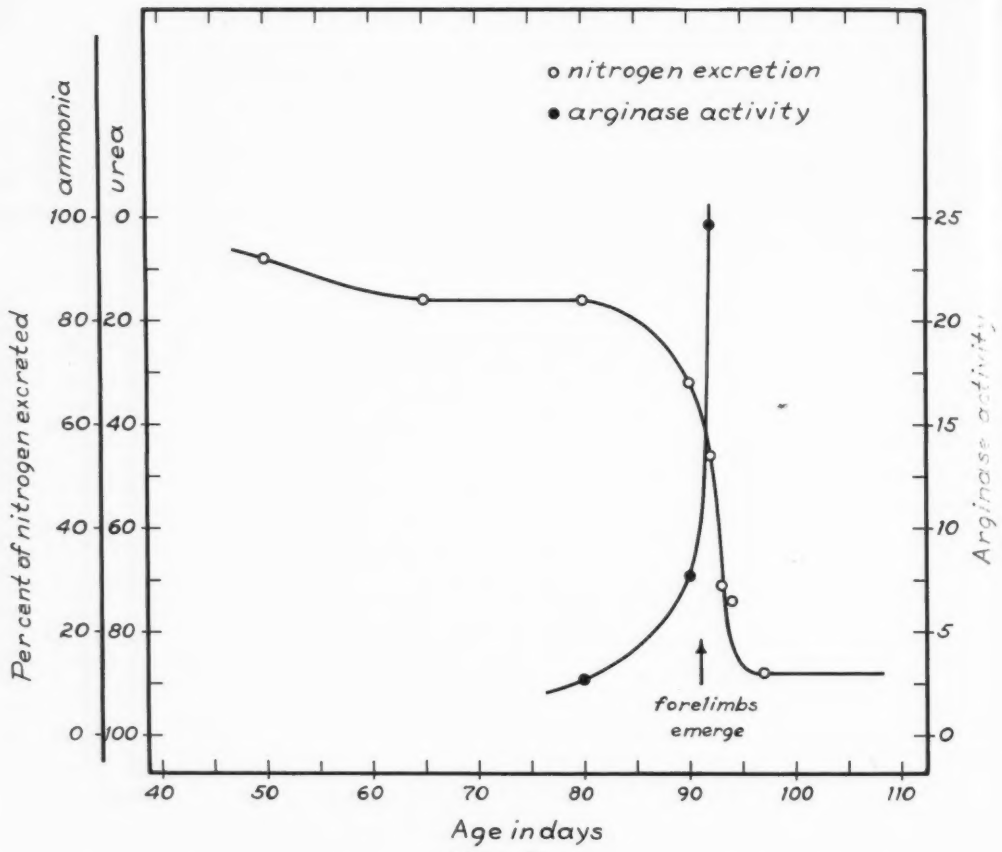


Figure 10

Biochemical metamorphosis of nitrogen excretion in the European frog, Rana temporaria. At the metamorphic climax, this animal changes from excreting about 90 per cent of its nitrogen as ammonia to excreting about 90 per cent as urea. Simultaneously, the activity of arginase, the enzyme that hydrolyzes arginine to form urea, rises sharply in the liver. (Drawn from the data of Munro.[21] Republished by permission of Academic Press.[10])

Indeed, *Xenopus* can change its mind again; for if kept moist while yet out of water it accumulates huge amounts of urea, perhaps as a device for conserving water such as is practiced by the elasmobranch fishes. Its return to water is attended by a massive excretion of urea accompanied by very little ammonia.[28]

We see, therefore, that even the aberrations of amphibian metamorphosis, anatomical and ecological, are paralleled closely by the biochemistry. It is probably true that in all cases in which the anatomy or the ecology changes, the biochemistry also changes. Indeed the biochemistry may have a primary status; the visible alterations in anatomy and ecology may only reflect prior biochemical changes.

Second Metamorphosis

The first requirement of a life cycle is that it be *circular*. Any organism that leaves its natal environment to explore, or grow up in, another must return at maturity to reproduce its kind. The spawning environment is fixed, whatever excursions animals may make as adults, and it is a truism that all animals must return to their natal environment to spawn.

For this reason, any animal that undergoes profound changes preparatory to migrating from its natal environment is likely to undergo a second series of changes *in the reverse direction* before returning. *Every metamorphosis invites a second metamorphosis.*

Let us begin with the common spotted newt mentioned above. This animal begins its life as an olive-green, gilled larva, living wholly in the water. After several months it metamorphoses to a lung-breathing, land-dwelling eft. The color changes to a brilliant orange-red, the skin becomes rough and dry, the lateral-line organs recede. The newt now lives 2 to 3 years wholly on land, growing meanwhile almost to full size. Then it undergoes a second metamorphosis: the color returns approximately to that of the larva, and the newt regains the wet, shiny, mucus-covered skin, the keeled tail, the functional lateral-line organs, though not, of course, gills. In this mature state it re-enters the water to spawn and live out the remainder of its life.[29, 30]

Many anatomical and behavioral aspects of the second metamorphosis can be induced prematurely in red efts by injection or implantation of anterior pituitary preparations;[30, 31] and a significant part of this complex of changes—the drive to re-enter the water, and the moult to a smooth, wet skin—is stimulated in hypophysectomized red efts by injections of prolactin, the lactogenic hormone of the anterior pituitary.[32]

I have already said that the mature animal possesses porphyropsin, like a fresh-water fish. These animals, however, had already undergone the second metamorphosis. Red efts on examination were found to possess mixtures of rhodopsin and porphyropsin, predominantly rhodopsin (fig. 11). The second metamorphosis in this species is accompanied therefore by the biochemical metamorphosis of its visual system from a predominantly rod type to that characteristic of fresh-water types.[10]

Recently, in extension of the present argument, Nash and Fankhauser[33] have examined

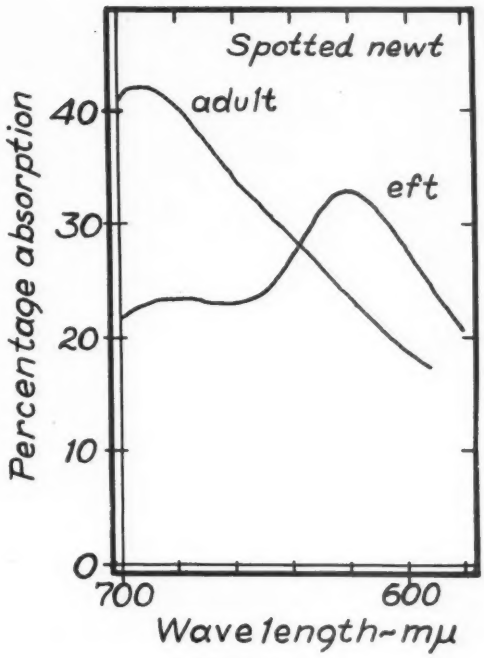


Figure 11

Second metamorphosis of visual systems in the New England spotted newt, Diemyctylus viridescens. Retinas of the land-living red eft contain a preponderance of vitamin A_1, with a minor admixture of A_2; retinas of water-phase, sexually mature adults contain vitamin A_2 predominantly or exclusively. (See Wald.[10])

the nitrogen excretion of this animal. Like other amphibia already mentioned, the larval newt excretes about 90 per cent of its total nitrogen as ammonia. At the first metamorphosis, it goes over to excreting urea, and the red eft excretes almost 90 per cent of its nitrogen in this form. Then, at the second metamorphosis, it turns back again, so that in the adults about one-fourth of the total nitrogen is excreted again as ammonia.

This, in turn, brings us back to the sea lamprey. This animal has a life cycle much like that of a salmon (fig. 12). After passing 4 to 5 years as a blind ammocoete larva, living buried in the sand or mud of its natal stream, it undergoes, while still in that position, a profound metamorphosis, preparatory to mi-

Figure 12

Development of the sea lamprey, Petromyzon marinus. This animal begins its life in streams as a blind larva, buried in mud or sand (stages 1 to 7). Then it undergoes a first metamorphosis while still in this position (stages 8 to 10), preparatory to migrating downstream. Several years later it undergoes a second metamorphosis, to the sexually mature adult, and migrates upstream again to spawn and die. (A, B) *Transformation of the mouth at first metamorphosis from the larval, hooded form* (L) *to the contracted circular form* (T). (n c) *Notochords of decayed adults found in streams after spawning. (Republished by permission of the New York State Conservation Department.*[34]*)*

grating downstream to the ocean or a lake for its growth phase. This lasts 1½ to 3½ years. Then the sea lamprey undergoes a second metamorphosis, to the sexually mature adult. The sexes differentiate visibly for the first time: the gonads mature, secondary sex characteristics appear, the males develop a rope-like ridge along the back, and either sex may assume golden mating tints. Then these animals migrate upstream to spawn.[34]

Some years ago I found that the sea lamprey, taken on its spawning migration, has almost exclusively vitamin A_2 in its retina, and I concluded from this that it probably possesses porphyropsin.[9] Since lampreys are members of the ancient class Agnatha, which includes the most primitive living vertebrates, I took this observation to support the view that porphyropsin is the ancestral type of visual pigment in vertebrates.

Recently, however, Crescitelli[35] reported that he had extracted rhodopsin from the retinas of this species and pointed out that this goes better with the opposed view, that rhodopsin is the primitive vertebrate pigment.

The specimens of sea lamprey examined by Crescitelli had just metamorphosed from the larval condition and had begun to migrate downstream, whereas the ones I had examined were at the other end of their life cycle, migrating upstream to spawn. On obtaining downstream migrants like Crescitelli's, I confirmed his observations exactly (fig. 13). The retinas of such animals contain vitamin A and

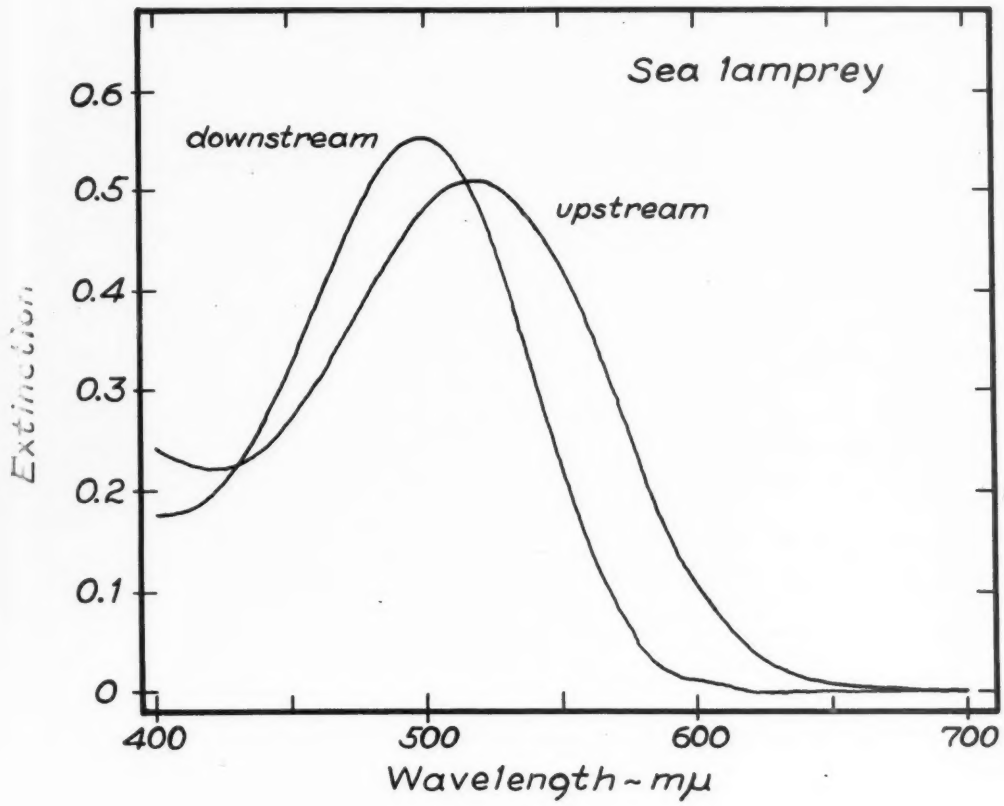


Figure 13

Second metamorphosis of visual systems in the sea lamprey, Petromyzon marinus. The downstream migrants, having recently undergone the first metamorphosis from the larval stage, possess rhodopsin. The sexually mature adults, migrating upstream from the sea to spawn, have porphyropsin alone. (From Wald.[36])

rhodopsin alone. The upstream migrants, however, possess vitamin A_2 and porphyropsin virtually alone.[36] We find therefore in this most primitive group of vertebrates another biochemical example of second metamorphosis, like that previously observed in the newt.

Such second metamorphoses expose fundamental characteristics of the metamorphic process:

1. Both the first and second metamorphoses *anticipate* changes in environment. Ordinarily they occur in the old environment and are completed there. They are *preparations* for the new environment, not responses to it.

2. Striking hormonal relationships are associated with these events. It has been known for many years that the first metamorphosis is stimulated in many instances by the thyroid hormone. A number of instances are now known in which phenomena associated with the second metamorphosis are stimulated by hormones of the anterior pituitary, including specifically the lactogenic hormone, prolactin.[30-32]

3. Just as the first metamorphosis prepares the animal to leave its natal environment, so the second metamorphosis prepares it to return, completing the life cycle. It is of the

essence of a second metamorphosis to reverse in part the changes which accompanied the first metamorphosis. The 2 metamorphoses tend to be opposed in direction, anatomically and biochemically.

4. Just as the changes in the first metamorphosis tend to have the character of recapitulations—that is, to coincide somewhat with the animal's evolutionary history—so the changes which occur in a second metamorphosis are likely to be antirecapitulatory, to reverse in direction the sequence of changes that accompanied the animal's evolution.

The last consideration involves a potential source of confusion. As I have already said, a life cycle is circular. If one section of it runs parallel with the course of evolution, another section is likely to run counter to that course. Just as every metamorphosis invites a second metamorphosis, so every associated recapitulation invites a subsequent antirecapitulation. This is only proper, provided it occurs at the point in the animal's history when it is being prepared for the return to the natal environment.

Deep-Sea Fishes; Eels

Heretofore I have discussed only changes in the visual pigments that involve their prosthetic groups. I should like now to discuss another type of change, involving the other component of a visual pigment, the protein opsin.

A short time ago Denton and Warren[37] reported that the visual pigments of deep-sea fishes, instead of having absorption maxima (λ_{max}) near 500 mμ, as do the rhodopsins of surface forms, have λ_{max} near 480 mμ. In consequence, they are orange in color rather than red, and Denton and Warren proposed that they be called chrysopsins, or visual gold. For reasons which appear below, I prefer to call them deep-sea rhodopsins.

This observation has since been confirmed by Munz[38] and by Wald, Brown, and Brown.[39] It makes good ecological sense; for the surface light that penetrates most deeply into clear sea water is blue, and made up of wavelengths near 480 mμ, and the rhodopsins of deep-sea fishes are more effective through having their maximal absorption in this region of the spectrum.[37]

As might be expected, the transition from surface to deep-sea rhodopsin is not sudden. A preliminary exploration shows that the absorption spectra of the rhodopsins shift more or less systematically with depth from the surface to about 200 fathoms (fig. 14). We find that throughout such a series the prosthetic group—the retinene—remains the same. It is the opsin which alters.[39] We have here a relationship comparable with that familiar in the hemoglobins, all of which possess the same heme joined with a variety of globins, different in every species.

Disregarding the relatively few rhodopsins and porphyropsins which lie in exceptional positions, one sees, therefore, a major transition from λ_{max} 480 to λ_{max} 500 mμ in the rhodopsins of marine fishes, correlated with depth, and depending on a systematic change of opsins; this connects with a further major transition from rhodopsin to porphyropsin (from λ_{max} 500 to λ_{max} 522 mμ) correlated with the transfer to fresh water, and depending on the change of chromophore from retinene$_1$ to retinene$_2$.

With this we can return to the "fresh-water" eel (*Anguilla*). Carlisle and Denton[40] have recently confirmed our observation that this animal, when taken in fresh water, ordinarily possesses the mixture of rhodopsin and porphyropsin described earlier; but they find that toward the beginning of its spawning migration it goes over to deep-sea rhodopsin (fig. 15). Whereas the absorption peak of its usual mixture of visual pigments, when the eel is in fresh water, lies at about 505 mμ, that of the animal about to migrate lies close to 485 mμ. Indeed, the rhodopsin of such a "fresh-water" eel preparatory to migration is virtually identical in spectrum with that of the permanently deep-sea conger eel.[41]

This is another instance of a second metamorphosis (fig. 16). The eel, having been spawned in the depths of the Sargasso Sea, journeys as a larva (leptocephalus) to the

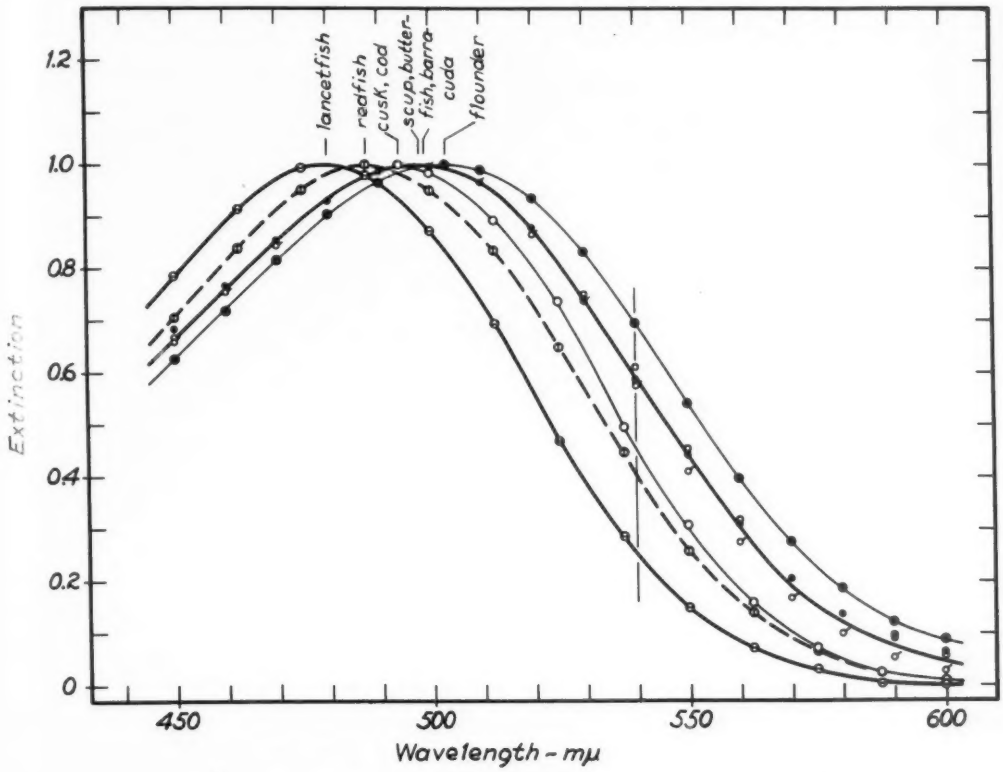


Figure 14

The rhodopsins of fishes taken at various depths in the sea. That of the lancet fish, found ordinarily below 200 fathoms, has λ_{max} about 480 mμ; those of surface forms (scup, butterfish, barracuda, flounder) have λ_{max} 498 to 503 mμ. The cusk and cod (from summer depths of 40 to 50 fathoms) have λ_{max} 494 to 496 mμ, and the redfish (from a depth of about 100 fathoms) has λ_{max} 488 mμ. (Republished by permission of Nature.[39])

shores of America or Europe.[43] There it metamorphoses to the adult form and usually, though probably not always, migrates into fresh water for its growth phase. Eventually it metamorphoses again: its color changes, the eyes approximately double in diameter, the digestive system deteriorates. As though getting ready for its return to the Sargasso Sea, it changes also to deep-sea rhodopsin.

My co-workers, Paul and Patricia Brown, have recently examined such animals at the Stazione Zoologica in Naples.* The European eel about to migrate seaward seems first to lose its retinal vitamin A_2 and retinene$_2$ and then to begin to combine vitamin A_1 and retinene$_1$ with a new, deep-sea opsin. The animal has already progressed far with this, as with the anatomical changes of the second metamorphosis, while still in fresh water.

Such observations can tell us something con-

*Recent experiments in our laboratory and at the Stazione Zoologica in Naples by P. K. Brown and P. S. Brown have demonstrated considerable variation in the proportions of vitamins A_1 and A_2 in the retinas of individual eels of both the American and the European species. These proportions vary between 65:35 and 25:75, with a mean value of approximate equality. The visual pigments, rhodopsin and porphyropsin, are present in approximately the same *molar* ratios; but since rhodopsin possesses a higher specific extinction than porphyropsin, extinctionwise rhodopsin tends to predominate.

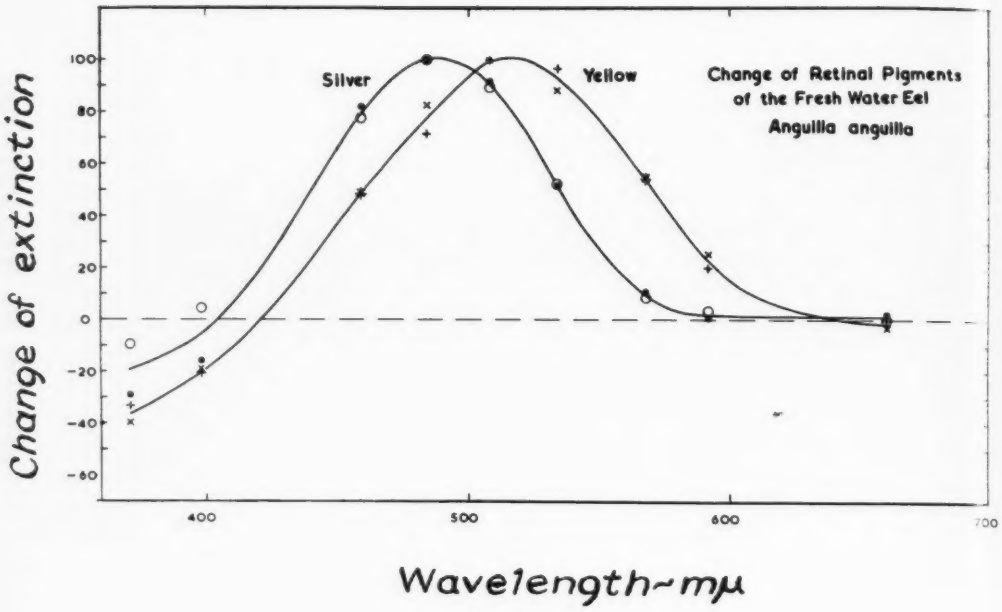


Figure 15

Second metamorphosis of visual pigments in the European "fresh-water" eel. The yellow eel, prior to second metamorphosis, has a mixture of rhodopsin and porphyropsin (net λ_{max} 500 to 505 mμ) corresponding to the mixture of vitamins A shown in figure 4. The sexually mature silver eel, about to begin its spawning migration to the Sargasso Sea, has changed over to deep-sea rhodopsin (λ_{max} about 487 mμ). (Republished by permission of the Journal of the Marine Biological Association of the United Kingdom.[40])

cerning the larval condition. To my knowledge, no one has yet examined the visual pigment of the leptocephalus larva, but the foregoing discussion suggests strongly that the pigment is deep-sea rhodopsin. Similarly, though no one seems as yet to have examined the retinal pigment of the larval New England newt, our observation that the adult at maturity metamorphoses to porphyropsin implies that this is also the larval pigment. Again, since the blind ammocoete larva of the sea lamprey metamorphoses to an eyed adult possessing rhodopsin, this is the first visual pigment to appear in this species. Yet the fact that in the second metamorphosis the pigment changes to porphyropsin implies that the latter represents the true, albeit missing, larval type. That is, since the second metamorphosis involves some measure of return to the larval condition, it can tell us something of the larval state, even of larval properties which have been lost in the course of evolution.

Land Vertebrates

Land vertebrates still pursue their embryogeny in water, but they have brought the water ashore. In a sense they are erstwhile amphibia which have carried water ashore in which their embryos go through the larval stages and first metamorphosis. They have developed 2 special devices for this: the boxed-in or cleidoic egg, and viviparity. Amphibia still experiment with both. Certain of them - for example, the American red-backed, slimy, and worm salamanders—lay eggs on land within which the larvae complete their entire development. Others—such as the European black salamander, *Salamandra atra*—retain the eggs in the body until the young are fully formed. The European spotted salamander,

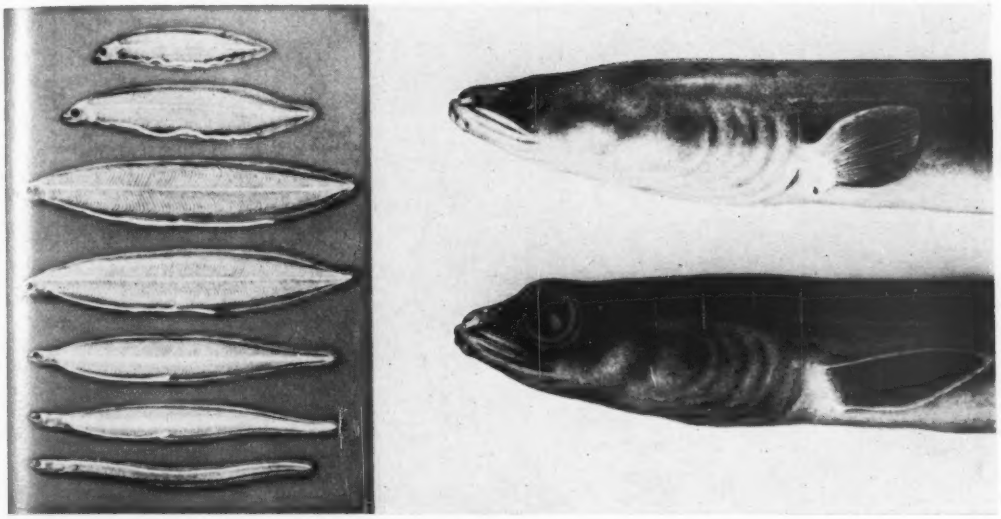


Figure 16

Development of the American eel, Anguilla rostrata. Left, top to bottom. *Three larvae, or leptocephali, of various sizes; then 3 larvae undergoing the first metamorphosis; and the metamorphosed "glass eel." The uppermost 3 pictures are enlarged 2.7 times relative to the others.* Right. *The second metamorphosis:* (top) *American eel in the "green" stage;* (bottom) *a European eel in the "silver" stage, ready for its spawning migration. Note particularly in the latter the approximate doubling in diameter of the eye. This large-eyed stage has not yet been observed in the American species. (Republished by permission of the Department of Fisheries, Province of Quebec.*[42])

S. maculosa, ordinarily lays its eggs in streams, but if it cannot reach water, permits them to develop internally.

One might hope, therefore, to find residues of metamorphosis in the embryogeny of land vertebrates, and in this one is not disappointed. Anatomical residues abound; they were the original source of the idea of recapitulation and were principally responsible for its early overexuberance. The embryo of a land vertebrate undergoes an anatomical metamorphosis approaching that of an amphibian. Unlike a larval amphibian, it never has functional gills; but, for a time, it does of course have gill slits, as well as other evidences of earlier aquatic life.

One finds biochemical metamorphosis also in the embryos of land vertebrates, and it includes some of the same changes with which metamorphosis in amphibia has already made us familiar.

So, for example, the chick embryo developing in the egg displays a changing pattern of nitrogen excretion which seems at first glance to mimic the evolutionary sequence (fig. 17). Joseph Needham's measurements,[44, 45] embodied in this famous figure, seem to show that the embryo begins by excreting about 90 per cent of its nitrogen as ammonia, like a fish; then about 90 per cent as urea, like most amphibia; and ends by excreting about 90 per cent as uric acid, as do adult birds.

This view by now needs reappraisal. Much of the impression conveyed by figure 17 comes from the fact that the data are plotted in terms of unit dry weight of the rapidly growing embryo. The recent measurements by Fisher and Eakin[46] of the amounts of these substances in the whole egg (fig. 18) show that the ammonia content remains almost constant, perhaps even falling slightly, throughout incubation. Ammonia seems never to be

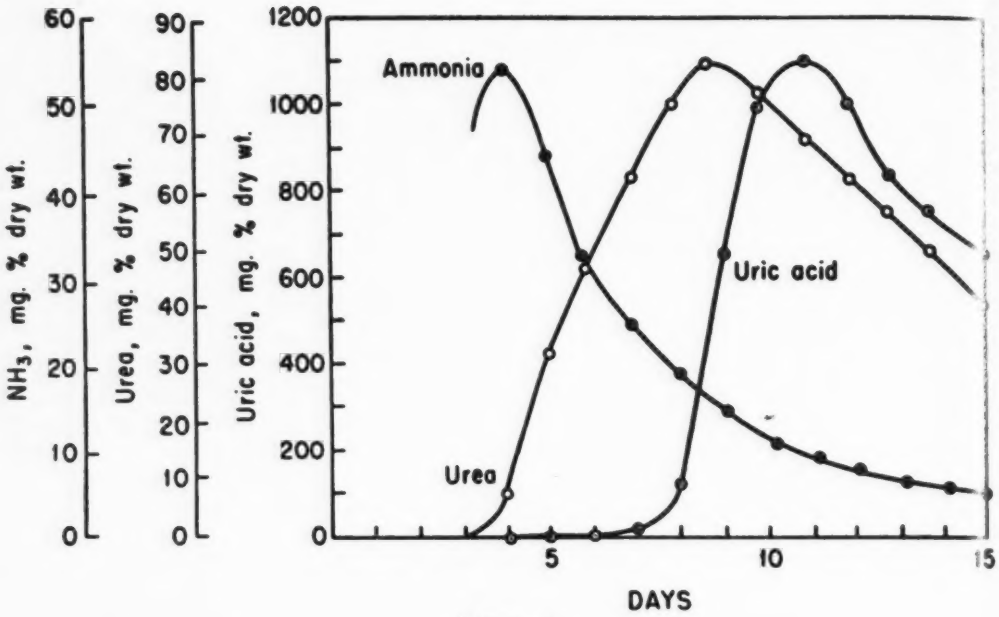


Figure 17

Measurements of ammonia, urea and uric acid in the developing chick, plotted in terms of unit dry weight of the embryo. (After Needham.) These measurements were originally interpreted to mean that the chick embryo first excretes about 90 per cent of its nitrogen as ammonia, then 90 per cent as urea, and finally 90 per cent as uric acid. When the data are plotted as here, the growth of the embryo is largely responsible for this appearance. Actually as figure 18 shows, in the whole egg ammonia remains almost constant in amount, urea steadily increases, and uric acid rises precipitately and continuously after the fifth to seventh day.

"excreted"; and its decline in concentration in figure 17 reflects only its dilution by the growing embryo. Urea increases regularly in amount, being not only formed, but excreted selectively into the allantois after the fifth to seventh day.[46] It declines in figure 17 only because its increase is overbalanced after a time by the still more rapid growth of the embryo. Needham, Brachet and Brown[47] showed that the sole source of urea is the hydrolysis by arginase of the arginine of the yolk. Urea is not formed from added ammonia, ornithine or uric acid; and this seems to be true also of the adult chicken.[47] That is, the chicken seems at no time to possess the enzymatic apparatus for synthesizing urea from ammonia and carbon dioxide. Of the enzymes of the ornithine cycle, it possesses only the terminal member, arginase.

What remains of biochemical metamorphosis in this account? I think 2 things:

1. A vestige of urea formation and excretion, connected with the presence of arginase in the early embryo. The arginase activity per unit weight of tissue declines rapidly, reaching very low values in the fifth to seventh day of incubation,[48, 49] owing in part to decrease in the concentration of the enzyme, in part to the formation of an arginase-inhibitor.[49]

2. The sudden institution, at about the same time, of uric acid synthesis and excretion, maintained throughout the further life of the organism.

A second example: In general, vertebrates hold the osmotic pressures of their body fluids

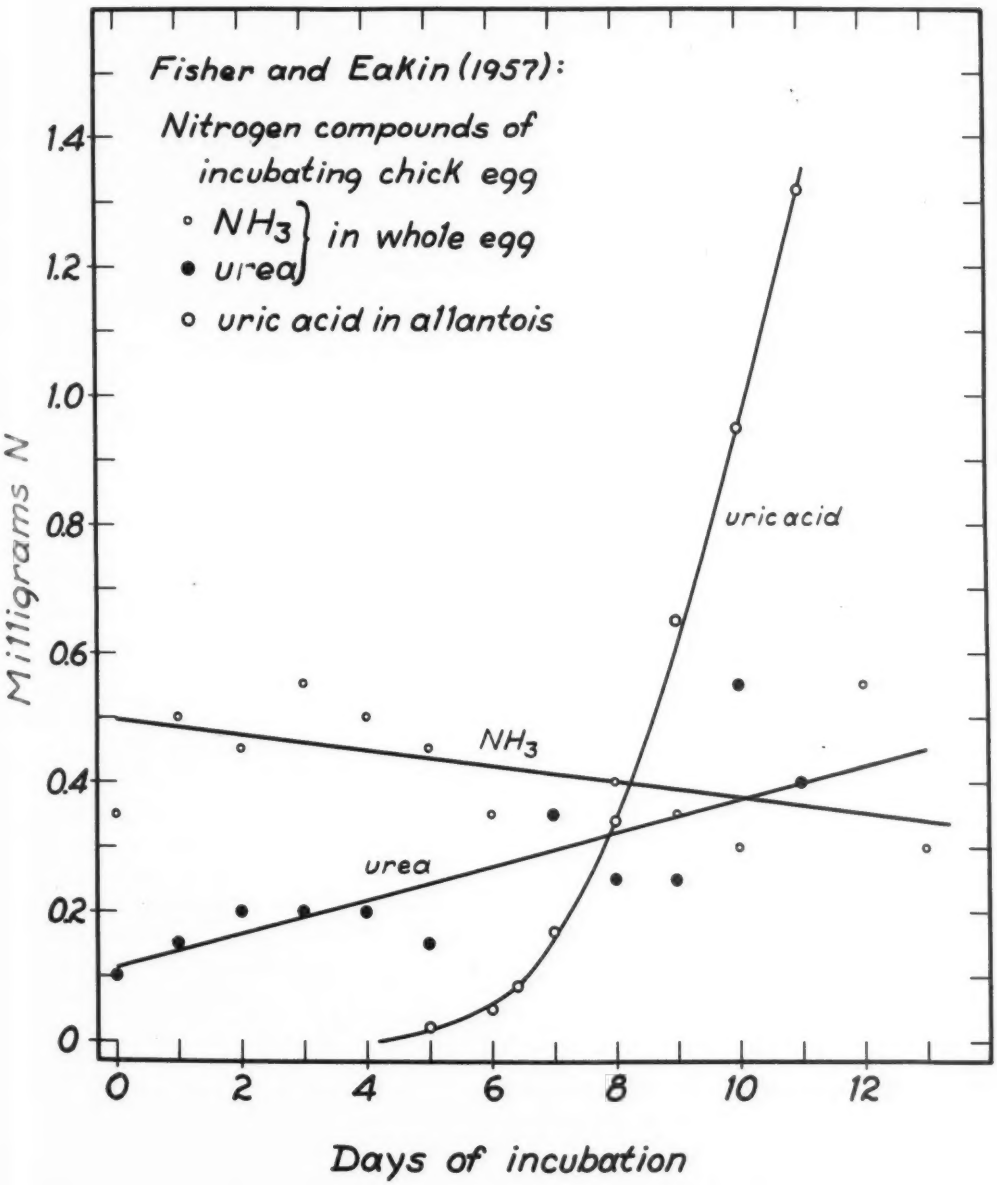


Figure 18

Ammonia, urea and uric acid content of the incubating chick egg. The ammonia content of the whole egg remains almost constant, perhaps declining slightly. The urea content slowly rises, and after a time (days 9 to 11) about half of it is excreted into the allantois. Uric acid appears in the allantois on about the fifth day, and thereafter accumulates rapidly. (Drawn from measurements by Fisher and Eakin.[46a])

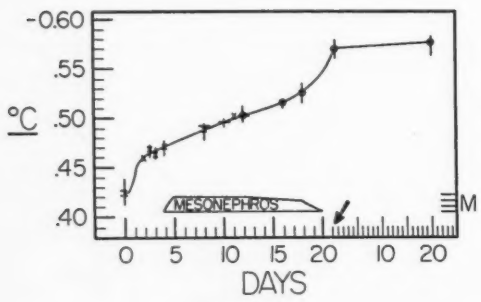


Figure 19

Biochemical metamorphosis of fluid osmotic pressure in the developing chick. Ordinates: *freezing-point depression, a measure of osmotic pressure.* Abscissae: *days of incubation, to hatching on the 21st day* (arrow), *and days thereafter;* (=) *unincubated egg white,* (×) *subgerminal fluids,* (—) *amniotic fluids,* (o) *bloods. The embryo begins with osmotic pressures characteristic of fresh-water fishes and amphibia and ends with the much higher osmotic pressure characteristic of mature birds and mammals. The duration of functional activity of the mesonephros* (M) *is also indicated. (Republished by permission of the Journal of Cellular and Comparative Physiology.*[50]*)*

within narrow limits. In the various groups of vertebrates the blood osmotic pressure takes characteristic values, correlated to a degree with the ecology, and perhaps also with the phylogeny (see [10]). Thus, the freezing-point depression (Δ F.P.) of the plasma in fresh-water fishes and amphibia lies at 0.45 to 0.55 C., whereas adult birds and mammals exhibit values of 0.55 to 0.65 C. Recent measurements in the developing chick[50] show that the fluids of the early embryo have freezing-point depressions of about 0.47 C. (fig. 19). The fluid osmotic pressure rises throughout development, with a final spurt at hatching that brings it to the adult value (Δ F.P. = 0.58 C.). That is, the embryo begins with a fluid osmotic pressure characteristic of fresh-water fishes and amphibia and ends with that characteristic of mature birds.*

A third example: Hall[54] has shown that during the embryonic development of the chick its hemoglobin changes radically, continuously losing affinity for oxygen, so that in an adult chicken more than twice as much oxygen pressure is needed for half-saturation as is needed in a ten-day-old chick (fig. 20). These changes persist for some time after hatching. They are similar in direction to the change in hemoglobin that accompanies metamorphosis in the bullfrog.

Comparable changes in hemoglobin accompany the embryonic development of all mammals so far examined. It is now well recognized that in mammals generally, man included, fetal hemoglobin is a different species of molecule from maternal or adult hemoglobin (see [55]) (fig. 21). Always—with the possible exception of man—the change in oxygen affinity is in the same direction, a loss of affinity as development progresses. The fetal and adult hemoglobins of mammals differ also in many other ways: in electrophoretic mobility, sedimentation rate, resistance to alkali, immunological specificity, solubility, crystal shape, and amino acid composition (for references see [10]). All these changes involve the globin moiety of hemoglobin; the heme is the same always.

The phenomenon of metamorphosis, biochemical as well as anatomical, extends therefore beyond the amphibia and fishes to include the land vertebrates, both egg-laying and placental.

Do land vertebrates exhibit also vestiges of a second metamorphosis? I suppose that puberty is so to be regarded. To be sure, this does not prepare a land vertebrate to

*This may be one example of a much more general phenomenon. In the frog *Rana temporaria*, the ovarian eggs have an osmotic pressure like that of adult blood (ΔF.P. = 0.41 C). Within a few hours after fertilization this has fallen to about 0.33 C., and in the gastrula stage reaches the extraordinarily low minimum of 0.275 C. Then it rises again, so that toward the end of the first week of development it again approaches the adult level (Krogh, Schmidt-Nielsen and Zeuthen;[51] Backman and Runnström;[52] Bialaszewicz[53]). I hardly know whether these changes in frogs and chicks are properly to be described as "metamorphoses." They may come too early in development and may be too continuous for that. I include them tentatively in this discussion in the hope that further examination will clarify their status.

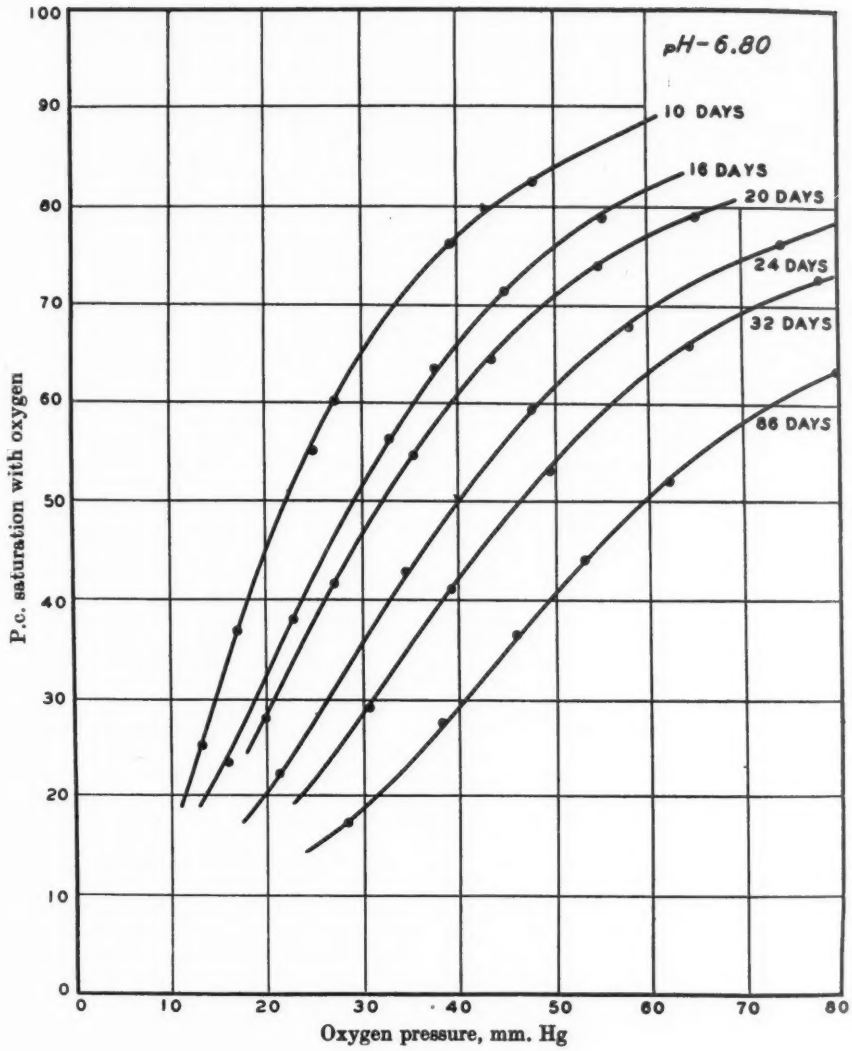


Figure 20

Biochemical metamorphosis of hemoglobin during the development of the chick. Measurements on dilute solutions of hemoglobin, buffered at pH 6.80, and equilibrated with oxygen at 37 C. The affinity for oxygen decreases regularly from the tenth day of incubation, and this change continues for some time after hatching. (Republished by permission of the Journal of Physiology.[54])

migrate, for the natal environment is now segregated, and puberty prepares the animal only to mate. Here only one representative cell—the spermatozoon—completes the return to the natal environment; and this, of course, undergoes a profound metamorphosis before being launched upon a migration as formidable, relative to its size, as that of any salmon.

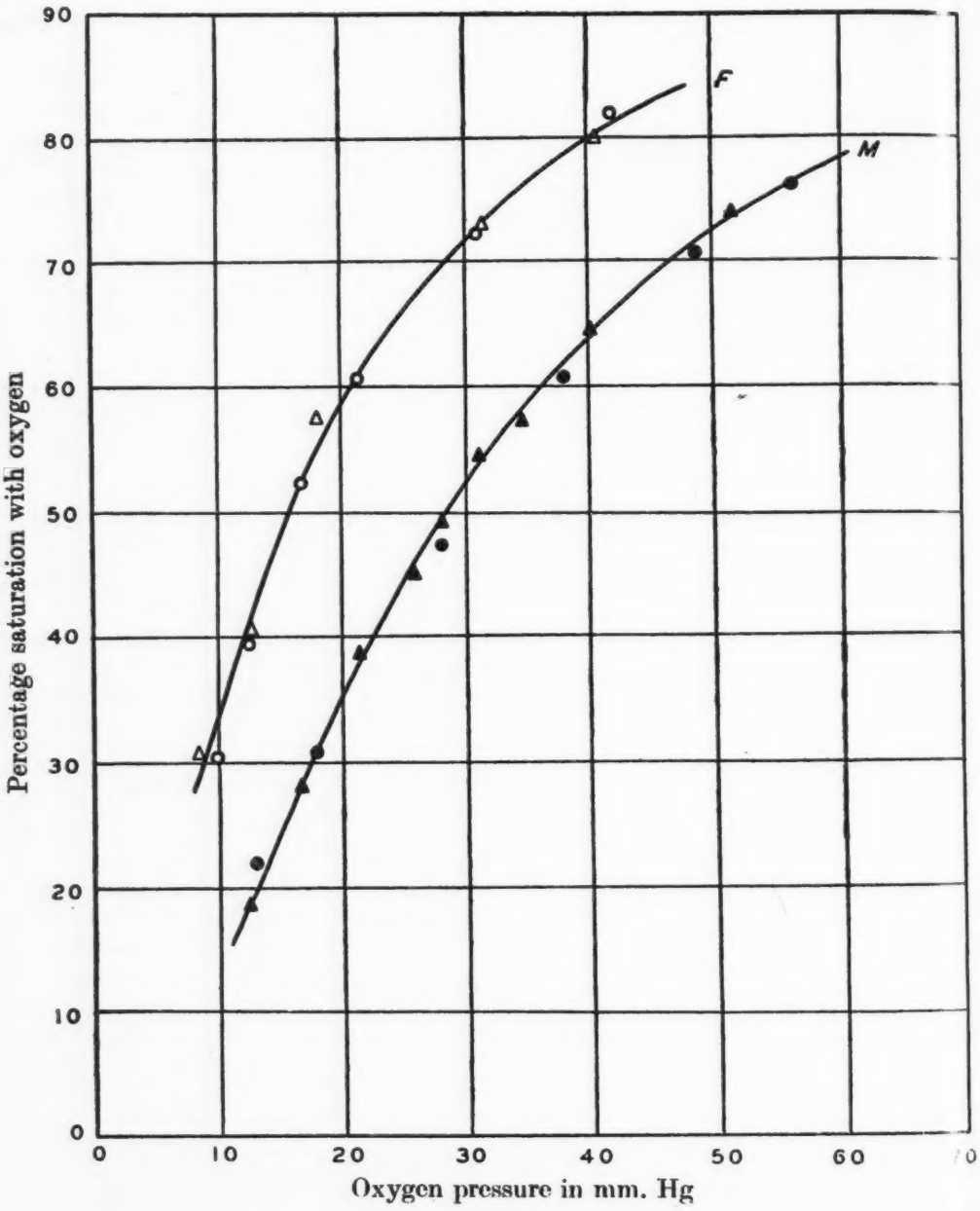


Figure 21

Biochemical metamorphosis of hemoglobin in a placental mammal. Oxygen equilibrium curves of hemoglobin from a goat fetus (F) *and from the mother* (M). *Both hemoglobins were obtained at 15 to 18 weeks' gestation, and measured in solution at 37 C. and pH 6.8. The fetal hemoglobin has almost twice as high an affinity for oxygen as the maternal hemoglobin. (Republished by permission of the Journal of Physiology.[55])*

Conclusion

Metamorphosis is a basic and general phenomenon, common to the whole vertebrate stock. It includes anatomical, physiological, and—perhaps prior to these—biochemical components, all designed to prepare the animal to leave its natal environment. Necessarily, in order to reproduce, the animal must eventually return, so completing its life cycle; and its return may be prepared for by a second metamorphosis, in some aspects the reverse of the first.

Our history as vertebrates is not dust to dust but water to water. From this point of view Nicodemus's great question can be given a broad and positive biological answer. Every animal can and must return to the "womb"—not, indeed, to be born again, but to bear the next generation. For a catadromous fish, the "womb" is the sea; for anadromous fishes and amphibia, a pond or stream; for land vertebrates, a uterus or egg. The question may raise additional problems only for man, and then only when the sense of return is toward the womb of the mother rather than that of the mate.

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IV. EXTRARENAL REGULATION OF SALT AND WATER METABOLISM

Chairman: John V. Taggart, M.D.

INTRODUCTION

By JOHN V. TAGGART, M.D.

In Dr. Smith's introductory remarks yesterday, he alluded to the tremendously rapid growth of renal physiology in the past 35 years, and we heard eloquent testimony to some of these developments in the program yesterday afternoon. During much of this period, the renal physiologists have been preoccupied with problems of salt and water, almost to the extent that, at times, the problems of salt and water have seemed to become their special province. And yet, we know that there is more to a river than its outflow. Today, we are going to move upstream, as it were, to tissues and sites more or less remote from the kidney, where salt and water metabolism are also of great importance.

Our first speaker, Dr. Saul Farber, is one of the few who have dared to penetrate those murky recesses of the body commonly referred to as connective tissue. He will consider the problems of salt and water metabolism in this ubiquitous tissue, with particular reference to the role of the mucopolysaccharides.

Our second speaker, Dr. Alfred Gilman, is well known to all of you. After many productive years as a renal physiologist, he has moved upstream virtually to the source, for during the past year he has directed his attention not to the kidney but to ion transport in the small intestine. I am sure that all of us are looking forward with keen anticipation to hearing this, his first presentation of the new data.

Dr. Knut Schmidt-Nielsen, our third speaker, is truly a peripatetic biologist. At times in the past, one could have found him in Arizona, studying water conservation in the kangaroo rat; later, he might have been found in the Sahara, studying the metabolism and thermoregulatory mechanisms of the camel. I first met Dr. Schmidt-Nielsen at the Mount Desert Island Biological Laboratory, where, several years ago, he began working on the bird commonly known in Maine as the shag, or cormorant. In this marine bird, he discovered a gland with such unique properties that it has excited the interest of everyone working in the field of electrolyte and water metabolism. The salt gland of marine birds promises, I think, to be a material of great biochemical interest as well.

Our fourth speaker calls our attention to ion movements within the eye. I think it is fair to state that the development of acetazoleamide brought workers in the fields of ophthalmology and renal physiology into a particular kinship. Dr. J. Everett Kinsey is a biochemist by training. He is the Assistant Director of Research at the Kresge Eye Institute in Detroit and also holds the rather

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unusual title of Professor of Ophthalmic Chemistry at Wayne University. I think, after you have heard his discussion, you will concede that this is an appropriate title.

Our final speaker of the morning, Dr. Henry Wheeler, will deal with a stream of salt and water which arises in the liver and then virtually disappears in the intestine. This much-neglected biological fluid, bile, is one which is proving to have many interesting features for anyone interested in salt and water metabolism. I hope that by the close of this paper, we will all be in a better position to appreciate some of the fascinating vistas of salt and water metabolism which are offered by certain of the extrarenal tissues.

Mucopolysaccharides and Sodium Metabolism

By SAUL J. FARBER, M.D.

This paper deals with the possible relationship between a native, negatively charged polyelectrolyte, chondroitin sulfate, and sodium metabolism. The physical chemical property of decreased activity of the cations Na, K and Ca in chondroitin sulfate is described. The data of experiments indicating an alteration in the mucoprotein metabolism of the cartilage of the rabbit's ear in response to the depletion of salt are presented.

SEVERAL years ago, we were interested in measuring the total exchangeable sodium and the body water in patients with heart disease. Total exchangeable sodium was determined by the dilution of radiosodium; body water was measured by the antipyrine dilution method. Patients with congestive heart failure and edema were studied before treatment and after cardiac compensation had been restored and edema could no longer be detected. The results demonstrated that there was an abundance of sodium in the body of the patient in congestive heart failure. An unexpected observation was the persistence of an increased body sodium in one-half of the nonedematous cardiac patients, i.e., after the edema was gone.[1]

The questions then arose of where the excess sodium could be, and why. To date there are no answers to these questions. In an effort to answer the question of where the sodium might be, one could postulate that the excess of sodium may be in cells, in bone or in components of the extracellular tissues. An interesting hypothesis is that excess sodium could exist in association with a polyelectrolyte as an osmotically inactive ion, a situation which would explain an abundance of sodium without equivalent amounts of water. Since connective tissue, or what is loosely called ground substance, contains polyelectrolytes with a high negative charge, we chose to study a polyelectrolyte component of such tissue. Hence, our studies concern chondroitin sulfate and a tissue rich in chondroitin sulfate, cartilage.

Cartilage Studies In Vitro

Beef nasal cartilage, a tissue very high in sodium and chondromucoprotein was chosen for this experiment. Thin slices of cartilage were placed in 2 cellophane bags. One bag was placed in a vial with H_2O and the other in a vial with KCl; the KCl solution was 70 μEq./ml. The outside solutions were changed every 2 hours and analyzed for Na, K and Cl (table 1). These experiments showed that cartilage can behave as an ion exchange resin.

The H_2O, or control, showed slight loss of Na and Cl from the cartilage. The outside solution of the KCl vial showed a decrease in K from 70 μEq./ml. and an increase in Na. Thus, over a period of 14 hours, there was an exchange of cartilage Na for K from the KCl, and the cartilage was converted from an Na to a K cartilage.

The cartilage was briefly washed and a solution of NaCl was placed outside the bag. Na now replaced the K in the cartilage.

Thus, the experiment indicated, as reported by Boyd and Neuman,[2] that cartilage can behave as an ion exchanger. The macromolecule in cartilage, chondromucoprotein, a high-negativity charged polyelectrolyte, cannot diffuse but can associate with exchangeable small counterions such as Na or K. These data, however, tell us nothing about the osmotic activity of the cations. To measure the activity of cations associated with chondromucoprotein and chondroitin sulfate, equilibrium dialysis studies were carried out.[3]

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Table 1

Dialysis of Slices of Beef Nasal Cartilage Concentrations in Dialysate

	H_2O μEq./ml. in dialysate			KCl μEq./ml. in dialysate concentration of KCl at the start of each period = 70 μEq./ml.		
Period	Na	K	Cl	Na	K	Cl
1	3.3	—	3.2	46.9	41.0	76.0
2	4.7	—	3.7	25.0	51.6	70.5
3	1.9	—	2.5	11.7	58.3	70.5
4				5.3	64.7	70.0
5				5.1	64.8	69.5
6				2.9	67.3	70.5
7				1.5	67.7	70.0
	Cartilage Washed; NaCl Dialysis Begun					
				μEq./ml. in dialysate concentration of NaCl at the start of each period = 70 μEq./ml.		
8				44.7	20.0	64.0
9				48.9	19.7	67.0
10				58.0	11.8	69.2
11				63.7	8.8	70.0
12				65.4	3.7	70.0
13				66.0	2.5	70.0

Each period was 2 hours of dialysis

Total μEq. Lost and Gained

Periods 1—7 Na lost = 1,337 μEq.
K gained = 1,051 μEq.

8—13 K lost = 978 μEq.
Na gained = 1,090 μEq.

Equilibrium Dialysis of Chondroitin Sulfate

Chondroitin sulfate isolated from beef nasal cartilage was used in equilibrium dialysis studies. The structural formula for Na chondroitin sulfate is shown in figure 1. The molecular weight of chondroitin sulfate (ChS) is about 20,000 to 30,000; the substance is a polymer consisting of 50 repeating units of the type shown in figure 1. Each unit consists of equimolar glucuronate and sulfated N-acetyl galactosamine. A counterion such as Na is associated with the carboxyl and sulfate groups. The salt of chondroitin sulfate is usually isolated as the Na, K or Ca salt.

Solutions of Na, K or Ca chondroitin sulfate were made in the respective chloride salt and dialyzed in cellophane bags against the respective chloride solution for 24 hours. After measuring the volumes, the pH and the concentrations of Na, K or Ca and Cl were determined.[3]

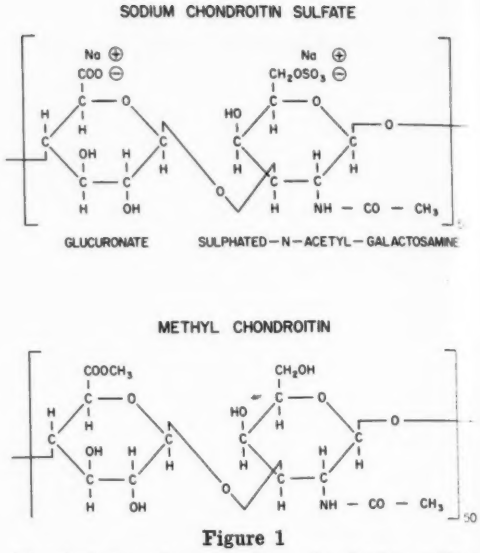

Figure 1

Chemical formulas of sodium chondroitin sulfate and methyl chondroitin.

If Na ChS were almost completely ionized, we would expect that the data obtained in an equilibrium dialysis experiment would obey the Donnan equilibrium. However, this was not the case; the product of the concentrations of Na and Cl inside the bag was always greater than the product of the ionic concentrations outside the bag. This indicated that a portion of the Na inside the bag was behaving as if it were not completely ionized. For the moment let us call this bound sodium. The bound sodium is calculated from the formulations:

At equilibrium inside the bag:

(a) Na^+i free + $ChS^{--} \rightleftarrows$ (Na^+i bound ChS^{--});

then:

(b) $$Na^+i \text{ free} = \frac{Na^+o \times Cl^-o}{Cl^-i},$$

(c) Na^+i bound = Na^+i total − Na^+i free.

The concentrations of Na and Cl inside (Na_i total), (Cl_i) and outside (Na_o), (Cl_o) the dialysis bag were determined. The free or completely ionized Na_i free, is calculated by dividing the product of the concentrations of the Na (Na_o) and Cl (Cl_o) outside the bag by the concentration of Cl (Cl_i) inside the bag

The bound Na can be obtained by subtracting the calculated free Na from the total concentration of Na inside the bag, value Na_i (total).

In this way, the equivalents of Na, K, and Ca behaving as bound cations could be determined and related to the concentration of ChS (equivalents bound per period ChS). Table 2 shows the data obtained for Na, K, and Ca at initial concentrations of cations varying from 0 to 140 μEq./ml. The data indicate that about one-half of the Na and K ions associated with ChS behave as if they are not ionized. Another characteristic of the polyelectrolyte is that, at this range of concentrations of cations, the amount calculated as bound is constant.

In order to establish the role of the carboxyl and sulfate groups in our system, methyl chondroitin was prepared. A methyl group replaces the Na and the sulfated N-acetyl galactosamine is desulfated (fig. 1). When methyl chondroitin is subjected to equilibrium dialysis there appears to be complete ionization of K and Cl inside and outside the bag. The concentrations of the ions are almost equal. There is no significant difference between the concentrations of K and Cl inside the bag (table 3).

The data indicate that the activities of Na, K, and Ca in a solution of ChS are decreased. This describes the data, but what could explain the decrease in activities? We can postulate that the observations are the result of a type of association or interaction which has been described by others as ion pair interaction or association. Thus, synthetic polyelectrolytes have been shown to have this property.[4-6] Snell has described a similar property for inorganic polyphosphates.[7] Tosteson has published data to indicate a decrease in the activities of Na and K by nucleotides.[8] Mathews titrated NaChS with .05 M HCl and found that increasing the NaCl concentration had the effect of decreasing binding of H^+ by chondroitin sulfuric acid. He interpreted his data as indicating a specific binding of H^+.[9]

Table 2

Summary of Cations Bound to ChS in a Concentration of 8.6 μperiods/ml.

Initial cation concentration μEq./ml.	Na^+	K^+	Ca^{++}
	Eq./Period of ChS		
10	.78	—	—
20	.79	.88	1.35
40	.93	.94	1.32
60	.92	.94	1.16
80	.77	1.01	1.50
140	—	.77	—
Averages	.85	.92	1.34

In summarizing this portion of the presentation, it may be concluded: (1) that a negatively charged polyelectrolyte native substance, chondroitin sulfate—a component of mucoprotein found in cartilage and ground substance—has the ability to maintain high sodium concentrations; (2) sodium chondroitin sulfate behaves as an incompletely ionized salt and therefore does not exert the expected osmotic effect of the measured concentration of Na.

Cartilage Studies In Vivo

Does mucoprotein play a role in sodium metabolism? It may be helpful to answer this general question before the possible role of the physical chemical properties of mucoprotein in vivo is explored.

The rabbit ear is a cartilaginous organ which is a small manufacturing plant of mucoprotein. There are a good number of cells in the ear producing mucoprotein, the metachromatic material found in cells and in extracellular matrix. As a model tissue rich in Na and mucoprotein, the rabbit ear is being used in assessing the role of mucoprotein metabolism in Na depletion.

The effects of the depletion of sodium on the electrolyte and chondroitin sulfate components of the cartilage of the ear were studied. Sodium depletion was produced in rabbits by injecting 5 per cent dextrose in water into the peritoneal cavity and removing the fluid 15 minutes after injection. The fluid removed from the peritoneal cavity contained Na, Cl,

Table 3

Mean Values for Ion Concentrations Inside and Outside the Bag at Several Concentrations of Potassium Chloride and with Methyl Chondroitin (5 mg./ml.) Inside the Bag

Concentration KCl at start μEq./ml.	No. of experiments	Concentrations at equilibrium				$\frac{K^+o \times Cl^-o}{K^+i \times Cl^-i}$ (μEq./ml.) Donnan equilibrium ratios
		K^+o	Cl^-o μEq./ml.	K^+i	Cl^-i	
20	2	14.6	13.8	14.8	13.0	1.05
40	2	28.0	26.3	28.2	25.9	1.01
60	2	40.5	39.3	42.1	38.7	0.98
80	2	54.1	53.2	54.4	52.1	1.02

Table 4

Weight, Ear Cartilage H_2O and Serum Electrolytes in Control and Na-Depleted Animals

	Wt.-Kg.*	Ear cartilage % H_2O	Serum Na	Serum K	Serum Cl
Control N=9	2.8	62	134	4.5	101
Na-depleted N=10	2.6	62	117	5.1	72
		Depleted†			
		Na	Cl		
		48	38		

*Wt.-Kg.=average weight of rabbits.

†Depleted=total mEq. removed from animals by peritoneal dialysis.

and very small amounts of K. The peritoneal dialysis was carried out 3 times in 1 day, and once more the following morning. Two to 3 hours after the final dialysis, blood was obtained by cardiac puncture and the animals were sacrificed. The ears were removed, the cartilage was freed from surrounding tissue, cut and weighed. The cut cartilage was homogenized in absolute alcohol, the alcohol decanted and the homogenized cartilage washed in ether. The decanted alcohol was evaporated and the residue analyzed for Na, K and Cl. These electrolyte values were added to those of the dried cartilage which was analyzed for Na, K, Ca, Cl and for mucoprotein constituents, hexosamine and sulfate. The results indicate that depletion of body Na leads to a decrease of Na in the cartilage of the ear.

The data in table 4 show the weight of the animals, the per cent of H_2O in the cartilage of the ear and the serum values for Na, K and Cl in sodium-depleted and in control animals. As anticipated, there was a decrease in the concentrations of Na and Cl in the serum of the animals which were depleted of sodium and chloride. At the bottom of the table are shown the quantities of Na and Cl in mEq. removed in the peritoneal dialysate.

Values obtained by analyzing the homogenized, dried cartilage of the ear are shown in table 5. There is a large decrease in the concentration of Na in the whole tissue amounting to 70 μEq./Gm. of dry tissue. To account for the Na in the extracellular fluid of the cartilage, the Na in the chloride space of the ear was calculated and subtracted from the total Na. The value for the decrease in the Na of the cartilage proves to be 43 μEq./Gm., a sizable decrease in Na. This decrease in Na probably represents Na associated with the mucoprotein of the cartilage. There is little, if any, change—certainly not an increase—in K or Ca, which could have indicated an exchange of other cations for Na.

Since there is no evidence for exchange of K or Ca for Na in the sodium-depleted animal cartilage, the possibilities remain that H^+ may exchange for Na or there may be a decrease in the mucoprotein or mucopolysaccharide of the cartilage. Titration data do not indicate a change in H^+ content of the cartilage.

In the event that a decrease in mucopolysaccharide took place, a decrease in hexosamine and, in particular, in sulfate would be found. Although there is a decrease in hexosamine, the error in the method is too large to make the difference significant. In paren

Table 5

Ear Cartilage Data in Control and Na-Depleted Animals

	Whole tissue	Cartilage				
	Na	Na*	K	Ca	Hexosamine	SO_4—μM
		μEq./Gm. Dry tissue				
Control N=9	439	246	144	87.6	199 (6.5)	211 (2.9)
Na-depleted N=10	369	203	134	84.6	184 (7.4)	194 (4.2)
Difference	70	43	10	3	15	17

*Cartilage Na=whole tissue Na minus extracellular Na as calculated from chloride space of cartilage.

Numbers in parentheses=standard error.

theses in table 5 are the values for the standard errors. The decrease in sulfate is statistically significant, but we are reluctant to accept the figures as absolute proof since the method is probably not sufficiently accurate for such a conclusion. The changes in hexosamine and sulfate, however, are of the order of magnitude to be expected, for the sum of the decrease in hexosamine and sulfate should theoretically add up to the decrease in sodium. (Each microperiod of ChS has 2 μEq. of sodium and in each microperiod of ChS there is 1 μEq. of hexosamine and 1 μM of sulfate.)

As a further attempt to show that sodium depletion affects mucoprotein metabolism, a study is now in progress which employs radioactive sulfate to label intracellular mucoprotein of ear cartilage.

It has been shown by radioautography that from 1 to 3 days following injection of S^{35} into a rabbit, most of the radioactivity in the cartilage of the ear is found in the cells.[10] In an effort to implicate intracellular mucoprotein in Na depletion, we took advantage of the intracellular localization of S^{35} radioactivity and performed the following experiments.

Pairs of rabbits were injected intravenously, at the same time, with the same volume of a solution of S^{35} sulfate. The next day, 1 of the pair of animals was dialyzed against glucose and water and the other was dialyzed against glucose in saline. Each animal was dialyzed 3 times. The second day after the injection of S^{35} another dialysis was performed, and 2 to 3 hours later the animals were sacrificed. Thus, we had a sodium-depleted rabbit and a control dialyzed rabbit; each had been injected, 2 days before sacrifice, with the same amount of S^{35}.

Table 6

Comparison of Total S^{35} Radioactivity Following Starch-Block Electrophoresis in Control and in Sodium-Depleted Animals

	S^{35} studies 2 days after injection CPM*		Ratio
Set	Control	Na-depleted	
1	619	465	.75
2	7,302	4,081	.56
3	1,920	1,430	.75
4	1,410	1,287	.91
5	1,796	1,255	.70
6	2,876	2,749	.95
7	1,824	1,239	.68
8	459	245	.53
9	2,913	1,153	.40
		Average	.69

*Total radioactivity (*CPM*) is the sum of counts under curves as shown in figure 2.

The cartilage of the ear was weighed, a volume of acetate buffer in proportion to the weight was added to the cartilage, and the tissue was homogenized. The homogenate was centrifuged at high speed, the supernatant was removed and dialyzed against an acetate buffer to remove free S^{35} sulfate. Starch-block electrophoresis was carried out in order to separate the mucoprotein from other constituents of the cartilage. Equal volumes of each dialyzed supernatant were applied to a starch block and electrophoresis was carried out in

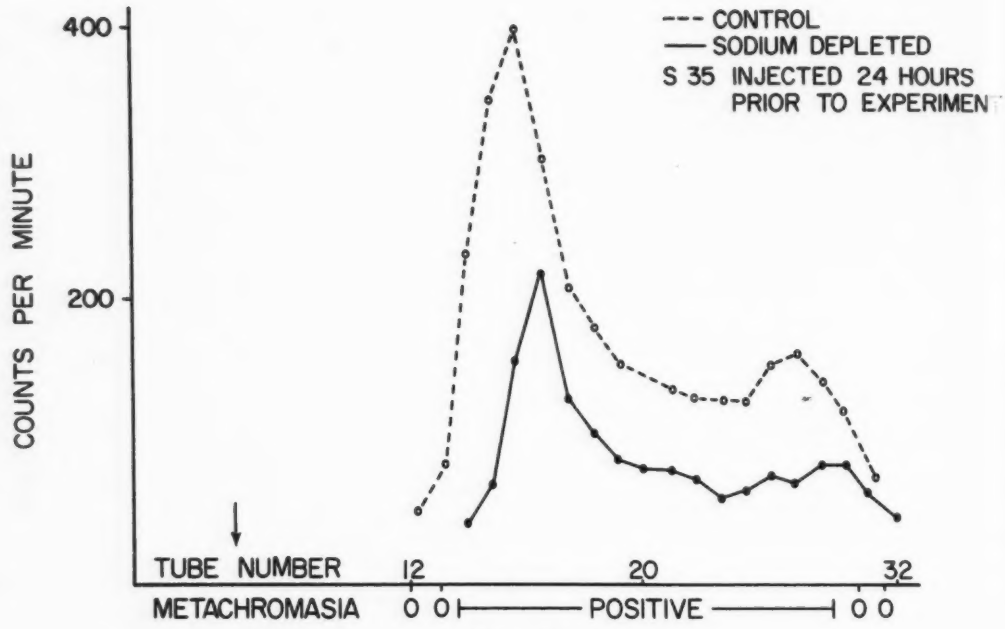

Figure 2

S^{35} radioactivity after starch-block electrophoresis. Metachromasia-positive indicates metachromatic stain with toluidine blue.

a phosphate barbital buffer of pH 7.0 for 12 to 14 hours. The starch block was cut into 5 cm. segments and each segment was eluted with a constant amount of buffer. After 100 λ of each eluate was pipeted into planchets, the radioactivity was counted. To detect metachromasia, the staining property of mucoprotein and chondroitin sulfate, 10 λ of each eluate was applied to a strip of filter paper and stained with toluidine blue.

The results of 1 such experiment are shown in figure 2. Along the ordinate are plotted counts per minute; along the abscissa are the tube number and the result of stain for metachromasia. The arrow indicates the tube number corresponding to the area of application of the supernatant. The results show that where there is S^{35} radioactivity, there is metachromasia, indicating that the S^{35} has been incorporated into the mucoprotein. There seem to be 2 components: one which is more radioactive, and another which travels faster but has less radioactivity. The broken line shows the data obtained from the control and the solid line shows the data obtained from the sodium-depleted animal. There is less radioactive mucoprotein in the cartilage of the sodium-depleted animal. Since 2 days after the injection of S^{35} the radioactivity is intracellular, we can assume that the data show a decrease in *intracellular radioactive mucoprotein.*

A series of 9 such experiments was performed in 18 animals, of which 9 were controls and 9 were sodium-depleted. Each animal of a pair was given the same amount of S^{35} sulfate intravenously and dialyzed (control 5 per cent dextrose in saline, experimental 5 per cent dextrose in water). Following final dialysis which was 2 days subsequent to S^{35} injection, the cartilage of the ear was homogenized, the supernatant was dialyzed and starch-block electrophoresis was performed. The sum of the counts under the curves of each control was compared to the sum of the counts of the corresponding sodium-depleted animal, and a ratio was calculated. The data appear in table 6. In 7 of the 9, the ratio was

below .9 and the average of all the ratios was .9. The data indicate that in sodium-depleted animals there is a decrease in intracellular radioactive mucoprotein. The observation does not necessarily mean that there is a decrease in *total intracellular* mucoprotein; the turnover rate may be altered. Experiments are now in progress to study the turnover rate of mucoprotein in sodium-depleted animals.

The data so far accumulated do not complete a hypothesis for the role of mucoprotein and chondroitin sulfate in sodium metabolism. The facts presented seem to be related but they do not fit smoothly into the jigsaw puzzle. However, we feel that the evidence indicates that: (1) cations of mucoprotein and chondroitin sulfate behave as if they are incompletely ionized; and (2) a tissue rich in sodium and mucoprotein participates in an abnormality of Na metabolism, namely, Na depletion. What these properties mean for human disease remains a challenging problem.

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Connective Tissue

Connective tissue is distinguished from other kinds in being richest in extracellular colloid materials, in having a close association with blood vessels—indeed, it serves as a support for blood vessels—and in exposing an enormous surface area. In such structures, chiefly, do the agencies rule which hold not only mobile water, but also substances dissolved in it, such as salts and sugar (glucose). Here few cells are found, but instead a "spongy cobweb of delicate filaments" which are held together by a small amount of "cement substance." Within the tiny interstices of this microscopic net of collagenous fibers are found protein substances (mucoid and small amounts of albumin and globulin). In this mesh, and bound by it in some manner, water and its dissolved substances appear to be stored. And it is in this mesh that the fluid collects when the heart or the kidneys fail to perform properly their functions.—W. B. Cannon. *The Wisdom of the Body*. New York, W. W. Norton & Co., 1932, p. 86.

Ion Transport in the Gut

By ALFRED GILMAN, PH.D., AND ETHOL S. KOELLE, B.S.

The in vitro preparation of the small intestine of the rat is well suited for studies of ion transport. Data from this preparation indicate that the passage of water and electrolytes across the intestinal epithelium depends on active transport. Glycolysis seems to be involved as a source of energy for the transport of ions from the mucosal to the serosal aspect of the intestinal wall.

LITERATURE on ion transport from the intestinal tract has expanded to the extent that a brief review of the subject must be limited to selected aspects. In the following discussion, attention will be directed primarily to the transport of ions from the small intestine of the rat, and major emphasis will be on data obtained from in vitro preparations.

Types of Preparations and Their Characteristics

The in vitro preparation of the small intestine of the rat is a highly desirable preparation for the study of ion transport for a number of reasons: (1) functional activity seems to be little affected by removing the intestine from the body if care is taken in handling the gut, and it is quickly transferred to a favorable environment; (2) transport is maintained with little diminution in capacity for several hours; (3) the amount of fluid transported is sufficiently great to permit an accurate analysis of its composition; (4) the composition of the fluid transported varies greatly in different portions of the small intestine, and this permits an approach to the study of individual transport systems; (5) the composition of serosal and mucosal fluids can be accurately controlled; (6) either net fluxes or bidirectional fluxes can be readily measured; (7) substrate requirements, the effects of specific inhibitors, metabolic characteristics, etc., can be determined with relative ease. The great disadvantage is that one is studying a mixed tissue. However, the contribution of the muscularis to total metabolic activity appears to be relatively small.

Three types of in vitro preparations have been employed, and each has advantages and disadvantages. In the "everted gut sac" a selected portion of the small intestine is removed and everted so that the mucosal surface is in contact with its external environment. A measured volume of fluid of selected composition is placed within the sac in contact with the serosal surface, and the ends are tied. The preparation is then placed in a suitable external medium. Changes that occur in mucosal fluid can be measured at intervals, and the volume and composition of the serosal fluid within the sac can be determined at the termination of the experiment. Small sacs can be conveniently studied in the Warburg apparatus.[1, 2] The main disadvantages relate to the extensive manipulation and the limitations in the size of the sac (volume of serosal fluid) when a number of analytic determinations are to be performed.

In another type of preparation, the lumen of any length or portion of the small intestine can be cannulated in vivo, washed, removed, and placed almost instantly in any oxygenated serosal fluid and perfused with an oxygenated, recirculating mucosal fluid.[3] Changes in composition of both serosal and mucosal fluid can be determined at any desired interval. The volume of serosal fluid can be sufficiently great to permit a relatively constant extracellular environment of the intestinal mucosa, but in the measurement of net transport and of the composition of the transported fluid analytic errors are exaggerated.

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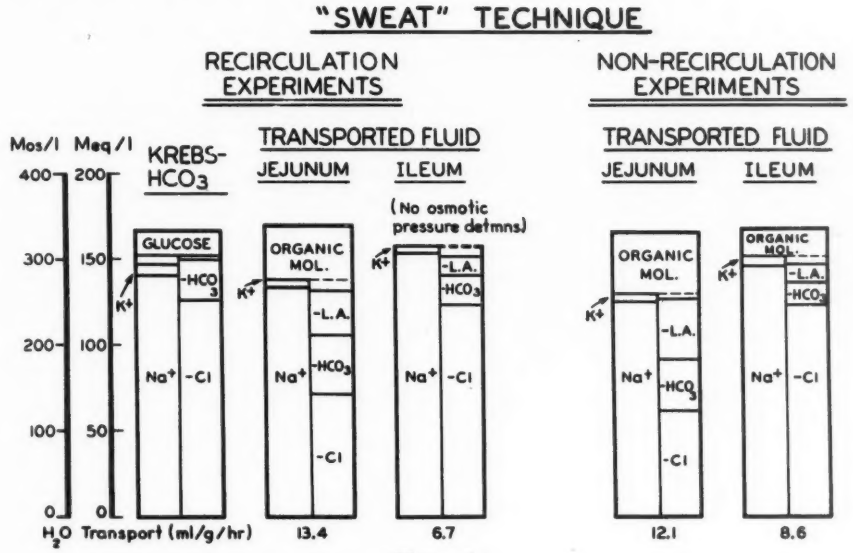

Figure 1

The composition of the fluid transported by the small intestine of the rat.

A preparation with oxygenated circulating mucosal fluid can be placed in a chamber in a gas atmosphere saturated with water vapor. Transported fluid appears on the serosal surface as droplets and collects at the bottom of the chamber.[4] The volume and composition of the serosal "sweat" can be accurately determined at any desired interval, and changes in the composition of the mucosal fluid can be followed simultaneously. The "sweat" technic is ideal for the study of the ionic composition of transported fluid. However, the composition of the extracellular fluid of the mucosal cells will approximate that of the transported fluid.

The authors have employed a modification of the "sweat" technic in which oxygenated mucosal fluid is not recirculated but is perfused from a reservoir at a rate of 5 to 10 ml. per minute. This provides mucosal fluid of constant composition and permits comparison of transport function over consecutive periods. The function of the gut remains remarkably constant for a period of 2 hours, and the effect of an inhibitor can be accurately assessed in the same piece of gut under control and experimental conditions.[5]

Composition of Transported Fluid

The composition of the fluid transported by the intestinal epithelium depends, of course, upon the contents of the mucosal fluid. Studies have been performed on the transfer of a wide variety of molecular and ionic species. Attention in the present discussion will be limited to the composition of serosal "sweat" when the mucosal fluid consists of Krebs-bicarbonate solution[6] containing 500 mg. per cent glucose. Only the major monovalent ions (Na^+, K^+, Cl^- and HCO_3^-) will be considered. In the balance of ions, lactate, which is produced in large quantity by the intestinal epithelium, must also be included.

Fluid transported by the jejunum differs greatly in composition from the Krebs-bicarbonate solution perfused through its lumen (fig. 1). Both fluids remain essentially isosmotic, but the total ion content of serosal "sweat" is less than that of mucosal fluid because glucose, which is actively transported by the jejunum, is present in the "sweat" in higher concentration. The "sweat" also differs in ionic composition. The concentration of chloride is much lower and that of bicarbonate higher than that of mucosal fluid. Lactate

Table 1

*Net Transport, Mucosal to Serosal Fluid**

	μEq./Gm./hr.					ml./Gm./hr.
	Na^+	K^+	Cl^-	HCO_3^-	Lactate$^-$	H_2O transport
Jejunum	1525	47	751	350	432	12.1
Ileum	1260	34	1060	110	95	8.6

*Average values of 11 experiments using jejunum and 5 experiments using ileum in which mucosal fluid is not recirculated and is of constant composition. Rate is expressed per gram dry weight of gut.

Table 2

*Composition of Mucosal and Transported Fluids**

	mEq./L.		
	Na^+	K^+	Total mOsm./L.
Mucosal fluid	105	6.2	327
Transported fluid	135	4.5	329

*The effect of the replacement of 40 mEq. of sodium chloride in mucosal fluid by an osmotic equivalent of mannitol. The transported fluid remains isosmotic with the fluid of origin. Since mannitol is not readily absorbed by the gut, a large concentration gradient relative to Na^+ develops.

Table 3

Effect of Various Substances in Maintaining Fluid Transfer by the Intestine†*

Substance	Initial concentration (mg./100 ml.)	Rate of fluid transfer (ml./hr.)
Glucose	500	6.0
	500	6.3
Galactose	500	0.95
	500	0.95
Fructose	500	2.2
	500	2.3
Sodium acetate	120	0.0
	120	0.0
Sodium propionate	148	0.0
	148	0.1
Sodium butyrate	176	0.1
	176	0.1
Sodium pyruvate	600	0.0
	200	0.0
	100	0.5
Sodium lactate	200	0.3
DL-Alanine	150	0.5
	150	1.7
	200	0.5

*The substances used were present in the mucosal fluid initially in the concentration shown.

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appears in both the mucosal fluid and the serosal "sweat" and displaces bicarbonate ion. Lactate ion is unevenly distributed between the 2 fluids. Much more diffuses into serosal "sweat" than into mucosal fluid. This phenomenon will be considered in greater detail subsequently. It will suffice to say that the true measure of the transport of bicarbonate into serosal "sweat" is represented by the sum of lactate and bicarbonate ions.

Ileal "sweat" is much different in composition than that produced by the jejunum. Indeed, when cognizance is taken of the displacement of bicarbonate by lactate, the fluid differs little in ionic content from that perfusing the mucosa.

There is also a significant difference in the total net transport capacity of the 2 sections of the small intestine (table 1). The rate of fluid transport by the jejunum is some 50 to 100 per cent higher than that by the ileum, depending upon the technic employed. This difference is due primarily to the greater net transport of bicarbonate by the jejunum. The rate of the transport of chloride is somewhat greater in the ileum than in the jejunum.

Evidence for Active Transport

There is little doubt that the passage of water and electrolyte across the intestinal epithelium results from active transport. No appreciable movement of fluid occurs in the absence of a suitable substrate or an adequate supply of oxygen.[4, 7-9] Furthermore, convincing evidence exists that energy is directed toward the transport of ions, and water moves along the osmotic gradient thereby created.[10, 11] There is no favorable electrical gradient for the movement of Na^+ ions, and a high concentration gradient of Na^+ can be achieved between serosal and mucosal fluid if the latter contains a molecular species such as mannitol which is not transported by the intestinal epithelium (table 2).

Substrate Requirements for Ion Transport

The substrate requirements for fluid transport by the isolated intestine of the rat are remarkably specific. Glucose, and to a lesser

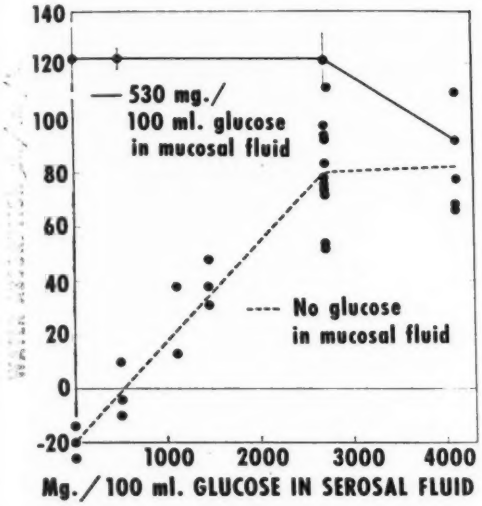

Figure 2

Effect of glucose concentration in serosal and mucosal fluids on rate of fluid absorption. (Republished by permission of the Proceedings of the Society for Experimental Biology and Medicine.[12])

extent fructose, promotes a high rate of transport. Various fatty acids and intermediates of carbohydrate metabolism fail to supply the requisite energy for maximal performance (table 3).[4] A puzzling observation is the fact that even when the gut is immersed so that its serosal surface is in a Krebs-bicarbonate-glucose solution, glucose must be present in mucosal fluid to be effective as a source of energy (fig. 2).[8, 9, 12] It is difficult to believe that the small intestine in vivo receives its energy for electrolyte transport by the simultaneous transport of glucose from the intestinal lumen. Yet this is certainly true of the gut in vitro. One could argue that glucose present in serosal fluid does not readily diffuse to the serosal poles of the mucosal cells. However, glucose in transport in the opposite direction readily gains access to serosal fluid. One cannot dismiss this observation lightly as an artifact of an "in vitro" preparation.

Source of Energy for Ion Transport

Glycolysis seems to be intimately involved as a source of energy for ion transport. The active transport of fluid from the small intestine can be markedly inhibited by agents which depress glycolysis. Table 4 depicts the observations of Smyth and Taylor[4] on the effects of various metabolic inhibitors on fluid transport. It is not surprising that substances such as 2,4-dinitrophenol and sodium cyanide interfere with active transport. Of greater importance to the present discussion is the marked inhibition of fluid transport by rela-

Table 4

The Effect of Inhibitors on the Transfer of Fluid by the In Vitro Intestinal Preparation†*

Inhibitor	Initial concentration (M)	Rate of water transfer (ml./hr.)
Phlorrhizin	2×10^{-3}	0.3
	2×10^{-4}	0.6
	10^{-5}	3.3
	2×10^{-6}	5.9
2,4-Dinitrophenol	4×10^{-3}	1.6
	10^{-3}	2.3
	10^{-4}	2.6
	10^{-5}	6.3
Sodium monoiodoacetate	10^{-3}	0
	10^{-4}	0.8
	10^{-5}	2.5
Sodium fluoride	5×10^{-2}	0
	5×10^{-3}	1.6
	10^{-3}	2.3
	10^{-4}	6.0
Sodium cyanide	10^{-3}	0.6
	10^{-4}	0.6
	10^{-5}	5.1
Sodium fluoroacetate	7.5×10^{-2}	2.6
	5×10^{-2}	3.2
	2.5×10^{-2}	4.5
	10^{-2}	6.4

*In the absence of inhibitors, the average rate of transfer is 6.0 ml./hr. In all cases glucose was present in the mucosal fluid in an initial concentration of 500 mg./100 ml.

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Table 5

*Net Ion Transport in the Jejunum (μEq./hr.)**

	Na^+	Cl^-	HCO_3^-	Lactate$^-$	HCO_3^- + Lactate$^-$
Serosal fluid	+915	+550	+199	+156	+355
Mucosal fluid	−900	−500	−412	+ 34	−378

*Net ion transport between mucosal and serosal fluid in the jejunum. Note the uneven distribution of lactate between the 2 fluids, and the fact that there is a large net transport of HCO_3^- into serosal fluid.

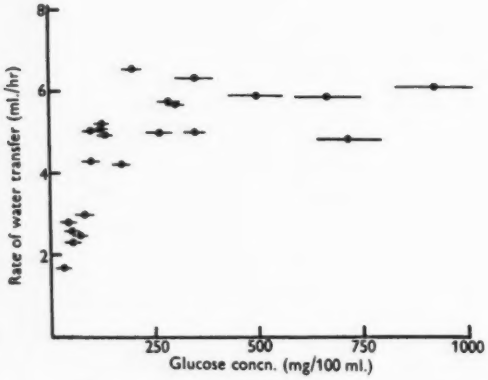

Figure 3

The relation between the rate of water transfer and the concentration of glucose in the inner fluid. Since the glucose concentration is not constant, the horizontal lines are used to indicate the probable limits of concentration. (Republished by permission of the Journal of Physiology.[3])

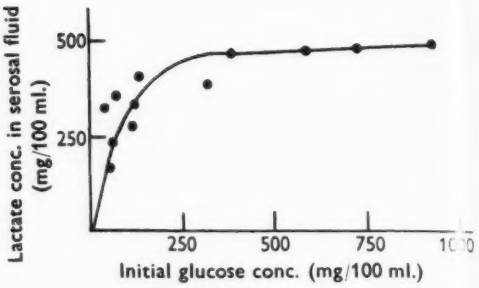

Figure 4

Relation between lactate concentration in the serosal solution and the initial concentration of glucose in the mucosal fluid. It can be seen that with increasing concentrations of glucose in the mucosal fluid, the concentration of lactate increases up to a maximum and then remains fairly constant. (Republished by permission of the Journal of Physiology.[3])

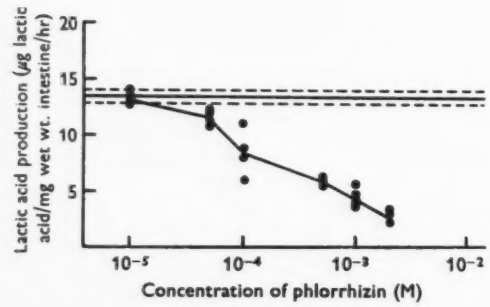

Figure 5

Effect of phlorrhizin on the production of lactic acid by sacs of everted intestine. The continuous horizontal line shows the mean value of lactic acid production in 8 experiments in the absence of phlorrhizin, and the 2 dotted lines are drawn at a distance above and below this equal to twice the standard error of the mean. (Republished by permission of the Journal of Physiology.[1])

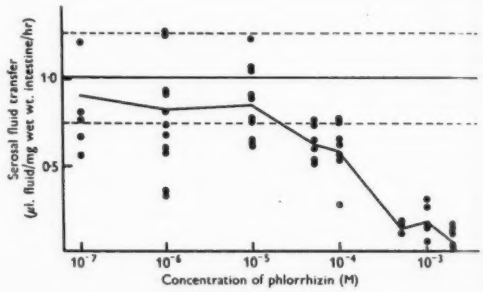

Figure 6

Effect of phlorrhizin on serosal fluid transfer. The points show the increase in volume of serosal fluid at various concentrations of phlorrhizin. The horizontal straight line shows the mean value for serosal fluid transfer in 14 experiments in the absence of phlorrhizin, and the 2 dotted lines are drawn above and below this at a distance equal to twice the standard error of the mean. (Republished by permission of the Journal of Physiology.[1])

tively low concentrations of iodoacetate, fluoride, and phlorrhizin which more specifically inhibit glycolysis. Not emphasized by Smyth and Taylor is the fact that fluoroacetate which acts specifically in the citric acid cycle has very little effect on the source of energy for transport. There is also a close correlation between glucose absorption, lactate production, and the rate of fluid transport (figs. 3 and 4).[4] Furthermore, agents which inhibit glycolysis (fig. 5) depress fluid transport (fig. 6) to a much greater extent than Qo_2 (fig. 7).[1] The support of ion transport by the glycolytic process has also been shown for other epi-

thelial membranes, notably the toad bladder and frog skin.[13, 14] Indeed, Leaf and coworkers have shown that there is sufficient glycogen in the bladder of the toad to support the transport of sodium in the absence of substrate and oxygen by anaerobic glycolysis, and that there is no large discrepancy between the efficiency of energy utilization by the sodium-transporting mechanism during aerobic or anaerobic metabolism.

Distribution of Lactate

As mentioned previously, lactate produced by the intestinal epithelium appears in much higher concentration in serosal than in mucosal fluid. This has been observed in the isolated bladder of the toad as well as in the intestine of the rat.[13, 15]

Wilson has suggested that the conversion of glucose to lactate may play a role in the absorption of glucose in vivo. Furthermore, he postulates that the disappearance of bicarbonate from mucosal fluid is due to the liberation of lactate ions on the serosal side, and of hydrogen ions on the mucosal side of the epithelial cells. He states that if lactic acid rather than lactate ion were liberated on the serosal side, complete displacement of bicarbonate ion would occur.[15]

There is little evidence to support this view, especially when one considers the net transport of ions that accompanies glycolysis. Table 5 depicts the large net transport of bicarbonate that occurs in excess of lactate production. Moreover, Leaf has shown in the bladder of the toad that the unequal distribution of lactate is influenced neither by the pH of the medium on either side of the membrane, by the transmembrane potential, nor by the concomitant transport of sodium ions.[13] He concludes that the asymmetric distribution is due to the fact that the mucosal surface is less permeable to lactate than the serosal surface and hence, more lactate diffuses through the serosal than through the mucosal surface.[16] Furthermore, in phosphate buffer solutions, no unequal distribution of the hydrogen ion from endogenous lactic acid could be demonstrated.[13]

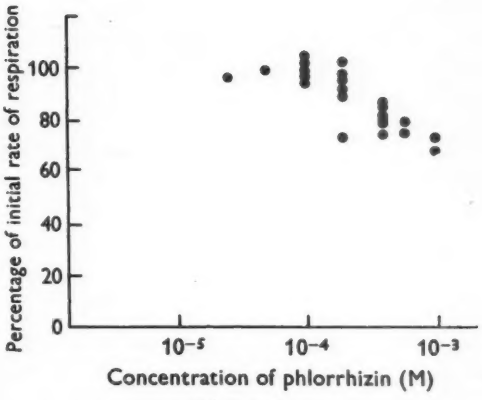

Figure 7

Effect of phlorrhizin on the rate of oxygen consumption of sacs of everted intestine. The points show the rate of respiration in the presence of phlorrhizin expressed as a percentage of the rate in the same sac before addition of phlorrhizin. (Republished by permission of the Journal of Physiology.[1])

It seems reasonable to conclude, therefore, that the jejunal mucosa actively transports ions from mucosal to serosal fluid. The composition of the transported fluid differs markedly from the mucosal fluid of origin (Krebs-bicarbonate solution) in that it has a higher concentration of bicarbonate and a much lower concentration of chloride. There is a large body of evidence which indicates that the energy for transport is provided by aerobic glycolysis. The large amount of lactic acid produced appears primarily in the serosal fluid where it displaces bicarbonate ion. However, bicarbonate transport far exceeds lactic acid production. Transport in the ileum appears similar in a qualitative sense, but there are marked quantitative differences.

In conclusion, it seems clear that the nature of the transport system in the small intestine, and indeed in any epithelial membrane, remains to be defined. The intestine of the rat in vitro offers a promising preparation for the elucidation of basic transport mechanisms. Functional capacity seems to be little compromised by placing the gut in an external environment, and the variety of technics available permits the study of transport of any

molecular or ionic species. To those who are unfamiliar with the preparation, exploitation of this relatively simple technic is highly recommended.

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The Free Life

What has the organism gained by constancy of temperature, constancy of hydrogen-ion concentration, constancy of water, constancy of sugar, constancy of oxygen, constancy of calcium and the rest? Is not the poikilothermic animal a very good animal? The bass or the perch with a blood on the acid side of normal, what fault is to be found with its muscular contraction or its heart beat?

. . . It is as a general rule the upper part of the central nervous system which suffers if the environment alters beyond physiological limits.—J. Bancroft. *Features in the Architecture of Physiological Function.* Cambridge, England, The University Press, 1934, pp. 79, 86.

The Salt-Secreting Gland of Marine Birds

By KNUT SCHMIDT-NIELSEN, PH.D.

Marine birds possess salt-secreting nasal glands which produce hypertonic solutions of sodium chloride in response to osmotic loads such as ingestion of sea water. The concentration of the secreted fluid is always high, several times as high as the maximum urine concentration in birds. The presence of this gland must be considered a necessary adaptation to marine life in animals whose kidney cannot excrete high salt concentrations. The structure and blood supply to the gland indicate a countercurrent flow, but at the moment it is not possible to explain the high concentrations of the secreted fluid as the result of a countercurrent multiplier system. The gland is under parasympathetic nerve control; its secretory function is blocked by anesthesia and certain drugs, including carbonic anhydrase inhibitors.

The Comparative Viewpoint

THE SUBJECT which I am going to deal with can, I think, be referred to as comparative physiology. Renal physiologists have more sophistication in comparative physiology than most other physiologists. They are used to discussing animals such as alligators and frogs and the very interesting and unique aglomerular fish, which has a kidney but no glomeruli. Names such as Marshall, Smith, Forster and others testify to the value of a comparative approach in renal physiology.

This leads me to say, without hesitation, something which might have seemed startling a few years ago, but, I am sure, will not be very surprising to most of this audience; that is, that the kidney is not always the most important organ of excretion, and, in fact, that the mammals constitute the only class of vertebrates in which the kidney is always the major organ of osmoregulation.

To explain this statement, it is helpful to review briefly the physiologic conditions for life in the sea and in fresh water. Earlier in this symposium Dr. Fishman quoted from Thales of Miletus that "Water is best," but he made the comment that salt is also good. To be specific, this is a matter of concentration. As you know, the ocean contains over 3 per cent salt, and the body fluids of all vertebrates contain around 1 per cent.* This poses serious physiologic problems of osmotic loss of water, as well as influx of salt from the more concentrated medium. In fresh water, the problems are reversed. Since the water contains very little salt, the fresh-water vertebrates face a steady influx of water and a loss of salt to the dilute surrounding medium.

Dr. Wald suggested yesterday evening that the vertebrates have evolved in fresh water. That may or may not be so. At this time I don't think we know, but it is convenient to start our discussion with fresh-water vertebrates. In fresh-water fish and Amphibia, the kidney is important because it eliminates, as a very dilute urine, the water which enters the body due to osmotic inflow. However, one could say that their major organ of osmotic regulation is not the kidney, for the fresh-water fish makes up for the loss of salts through active uptake of ions in the gill, and the amphibian has a similar mechanism in the skin. In fact, the frog skin has become a classical object of study because it is so eminently suited for experimental work on ionic transport mechanisms, and has permitted the

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*The most primitive vertebrates, the cyclostomes, are exceptions; they are in osmotic equilibrium with and have salt concentrations similar to sea water. The elasmobranchs are in osmotic equilibrium with sea water, but a major part of the solutes in their body fluids is urea and trimethylamine oxide, and the salt concentration of their body fluids is similar to that of other vertebrates.

development of Ussing's ingenious method of measuring the ion flux by means of the short-circuit current.

Salt-water fish have an osmotic loss of water to the concentrated surrounding medium and compensate by drinking sea water. The excess salt is eliminated by the pump in the gill, which, compared to the fresh-water fish, has been turned around. Again, we have a case in which the kidney is not the major organ of osmoregulation. No amphibian has been reliably reported to live in salt water, and it seems that the amphibians as a group are unable to solve the physiologic problems of adaptation to life in salt water.

Other vertebrates, reptiles, birds and mammals are, of course, mostly terrestrial. In these the kidney is the major organ of excretion, and, as such, a very efficient organ. The mammals, in particular, have evolved a kidney with a high concentrating ability, which, as Marshall pointed out, undoubtedly is related to the presence of the loop of Henle. More recently, the role of the loop structure has become clear with the understanding of how it functions as a countercurrent multiplier system.

Some mammals, birds and reptiles have evolved a secondary adaptation to a marine habitat. Seals and whales can handle the problem of life in salt water because their kidney can produce a highly concentrated urine. Elimination of the salts from sea water is well within the capacity of the mammalian kidney. Desert rodents, such as kangaroo rats and jerboas, which have to conserve water, have kidneys that can excrete urine more than twice as concentrated as sea water. The reason that sea water is toxic to man is primarily that his kidney is not very efficient in comparison to other mammalian kidneys.

The kidneys of birds and reptiles have a concentrating ability which is considerably less than that of the mammalian kidney, apparently because they contain no typical loops of Henle. Birds, which can excrete a urine which is about twice as concentrated as the plasma, have some looped nephrons, but these are not as highly developed as in mammals. Reptiles have no loops in their kidneys, there is no countercurrent multiplier system, and their urine cannot be concentrated above the plasma concentration. As a consequence, marine birds and reptiles cannot rely on the kidney for osmoregulation, and they appear to have a choice between avoiding the drinking of sea water and acquiring some means for salt excretion more efficient than their kidneys.

Marine Birds

Many birds and a few reptiles have adapted to marine life. Some birds come to land only to breed, and spend most of the year on the ocean; others, such as the penguins, have become excellent swimmers and have lost the power of flight. The emperor penguin is said never to come on land, not even to breed, but this statement is merely a play on words, for the emperor penguin hatches its eggs standing on the antarctic ice during the cold polar winter. Many marine birds eat fish, which contain much water; this circumstance reduces their problem of salt excretion. In fact, on a diet of fresh fish, cormorants have plenty of water to spare and do not need to drink at all.[1] But other birds eat invertebrates which are in osmotic equilibrium with sea water; gulls eat mussels, sea urchins and crabs, eider ducks filter small organisms out of the water, some penguins eat large quantities of krill, and so on. How do these birds, with their inefficient kidney, eliminate the salt that is present in their food and in the water they may drink? The answer is that they possess an accessory organ, a gland in the head, which produces a concentrated salt solution that drips off from the tip of the beak.

This salt-secreting gland is highly developed in all marine birds, as opposed to terrestrial birds. The gland has long been known to anatomists as the nasal gland; it is present in all birds, but in terrestrial species it is very small.[2] The size of the gland in marine birds is 10 to 100 times as large, a striking difference that was noticed long ago. The

ual interpretation has been that the function of the gland should be to rinse away the irritating and harmful effects of sea water that might penetrate into the nasal cavity. However, in all the marine birds that we have examined, the gland secretes a solution of sodium chloride which is more concentrated than sea water, and the exclusive function of the gland seems to be that of osmoregulation. The species which we have examined, which include gulls, terns, auks, penguins, albatrosses, petrels, cormorants, pelicans, gannets, herons and ducks, represent all the major orders of marine birds; it seems logical, therefore, to conclude that the salt-secreting gland is present in all marine birds.

It is interesting that the gland not only is large in marine birds, but that, to some extent, its size varies with the exposure to salt loads. Long before the function of the gland was known, Schildmacher[3] found that ducks brought up with access to a 3 per cent salt solution instead of fresh water developed an enlarged gland. However, after a careful discussion of the apparently causal connection between the size of the gland and salt water, Schildmacher repeated the mistake of the old interpretation, stating that the only possible explanation is that the gland protects the nasal mucosa against irritation.

The change in the size of the gland with the degree of exposure to salt was observed earlier by the Heinroths, who found that eider ducks which were reared in fresh water in the Berlin Zoo had smaller gland impressions on their skulls than individuals of the same species collected in the wild.[4] However, the relative ease with which the gland hypertrophies with use and atrophies with disuse should not be taken to mean that terrestrial birds can develop a functional salt gland from their nasal gland. This does not seem to be the case; only those birds that have an evolutionary history of adaptation to a marine habitat seem to have evolved a gland of significant size, and seem to be capable of secreting hypertonic solutions.

With the apparently universal existence of the salt gland in living marine birds, its presence in fossil forms can safely be taken as an indication of a marine habitat. Some fossil birds, such as *Ichthyornis* and *Hesperornis* from the Cretaceous period, have clear impressions on the skull where a large gland has been located.[5] These birds were fish eaters, and supposedly marine, and the presence of the large gland in the same location as the salt gland of living birds is a satisfactory confirmation of their marine life.

Marine Reptiles

Only a few reptiles live in the sea; they represent 4 orders: turtles, snakes, lizards and crocodiles. The great sea turtles eat fish or seaweed, and go on land only to lay their eggs on sandy beaches. They are known to shed tears as the eggs are deposited, evidently a sign of osmoregulation, rather than pain.[6] The sea snakes (Hydrophidae) of the Indian Ocean are truly marine; the more primitive lay their eggs on land, but the most specialized bear living young and remain at sea throughout life. They are reputed to be the most poisonous of snakes, and their osmoregulation has never been studied. A single lizard, the Galápagos lizard (*Amblyrhynchus cristatus*) is marine; it lives on the beaches of the Galápagos Islands where it eats seaweeds and occasionally blows a spray of droplets out through its nostrils, reminiscent of a puff of smoke from a fiery dragon. This harmless creature is easily handled in the laboratory, and it proves to have an osmoregulatory nasal gland that produces the salty fluid which is ejected from the nose. The marine crocodile (*Crocodylus porosus*) has been found far out at sea, but normally it lives in estuarine swamps. A single specimen which I had in the laboratory did not respond to osmotic loads, and crocodile tears must still be classed as one of the mysteries of biology.

Terminology

The presence, but not the function, of the salt-secreting gland has been known for centuries[7] and received considerable attention early in the last century.[8–10] It has been called

the nasal gland (glandula nasalis), although it is not always found in what one would call the nose. In most marine birds it is located on top of the head, above the orbit of the eye, and has therefore also been called the supraorbital gland, or glandula nasalis supraorbitalis. In the marine turtles the salt-secreting gland is seemingly of a different embryologic origin. It is located in the posterior part of the orbit of the eye, and its duct opens in the posterior corner of the eyelids. This gland in the turtle should, therefore, probably be regarded as a modified lacrimal gland. It is interesting that the gland of the turtle, in spite of its different origin, has the same histologic structure as the gland of the bird.

Since these glands, which anatomically are not homologous, have the same function, the need arises for a convenient terminology. We have therefore decided to call them salt-secreting glands, or simply salt glands; this designation, then, denotes any gland in the head region of marine birds and reptiles which, irrespective of anatomic origin, has an osmoregulatory function and secretes highly hypertonic sodium chloride solutions.

Response to a Salt Load

The effect of a considerable salt load in a black-backed gull (*Larus marinus*) is shown in table 1. In this case the bird, which weighed 1,420 Gm., was given almost one-tenth of its body weight of sea water by stomach tube. After about 3 hours the total volume of the excreta equaled the infused amount, and all the ingested salt had been eliminated. In other words, a gull handles quite easily a salt load which could not possibly be tolerated by man.

Some details of the relative importance of the kidney and the salt gland are given in table 2. The total amount of fluid excreted from the salt gland was 56 ml., while in the same 3-hour period 75 ml. was eliminated from the cloaca, most of this latter being urine. The concentration of sodium was uniformly high in the nasal fluid, while it was low in the cloacal fluid. Thus, the total amount of salt eliminated by the salt gland was about 10 times as high as the total cloacal excretion. In other words, in this particular experiment, the extrarenal excretion of salt was 10 times as important as the renal excretion. In spite of the salt load, the concentration of sodium in the urine was low and tended to decrease further during the experiment, although the kidney has the capacity to excrete concentrations of sodium up to about 300 mEq./L. This drop in the concentration of sodium in the urine after a salt load has frequently been observed in gulls, but in other species of birds about which we have similarly complete records of the effects of a salt load, the concentrations of sodium in the urine have tended to rise toward the concentration ceiling (about 300 mEq./L.).

Composition of the Nasal Secretion

The fluid secreted from the salt gland is very simple. Except for epithelial cells and other debris in samples collected immediately after the start of secretion, the almost neutral, watery liquid is clear and colorless. The dominating solutes are sodium and chloride in approximately equivalent amounts (table 3). There is a relatively small amount of potassium, some bicarbonate, and almost nothing else. Magnesium and sulfate, which are present in sea water in rather high concentrations, are virtually absent from the nasal fluid. The amount of organic material, including urea, is very small. Phenol red, which apparently is excreted by all kidneys, is not secreted by the nasal gland and does not appear in the fluid.

Concentration of the Fluid

The activity of the salt gland is an all-or-none phenomenon. If there is an osmotic load, the gland secretes; in the absence of an osmotic load, the gland is at complete rest. In this intermittency, the gland differs from the kidney which produces urine continuously. The concentration of salt in the nasal secretion is always very high, and remains fairly constant for each species (table 4). There seems to be a clear connection between the concentrating ability of the salt gland and

Table 1

The Total Excretion of Water and Salt in a Black-Backed Gull After a Load of Sea Water by Stomach Tube

Body weight	1,420 Gm.
Sea water ingested	134 ml.
Excreta, total volume in 3 hrs.	131 ml.
Sodium ingested	54 mEq.
Sodium excreted in 3 hrs.	48 mEq.

Table 2

Nasal and Cloacal Excretion by a Black-Backed Gull During the 175 Minutes Following the Ingestion of Sea Water (see Table 1)

	Nasal excretion			Cloacal excretion		
Time, min.	Vol., ml.	Sodium conc., mN	Sodium amount mEq.	Vol., ml.	Sodium conc., mN	Sodium amount, mEq.
15	2.2	798	1.7	5.8	38	.28
40	10.9	756	8.2	14.6	71	1.04
70	14.2	780	11.1	25.0	80	2.00
100	16.1	776	12.5	12.5	61	.76
130	6.8	799	5.4	6.2	33	.21
160	4.1	800	3.3	7.3	10	.07
175	2.0	780	1.5	3.8	12	.05
Total	56.3		43.7	75.2		4.41

the feeding habits of the bird. For example, in the cormorant, which eats fish that is relatively low in salt content, the concentration in the secretion from the gland is about 500 to 550 mEq./L. and rarely exceeds 600 mEq./L. In the herring gull, which consumes more invertebrate food, and consequently more salt, the concentration is usually between 600 and 800 mEq./L.; the great black-backed gull which is more marine in its habits, has concentrations between 700 and 900 mEq./L. The petrel is a bird with pronounced oceanic habits. It spends most of its life at sea and comes to land only to breed. It lives on planktonic organisms, mostly crustaceans, which it picks off the surface of the ocean as it flies by. The planktonic organisms, being invertebrates, have the same osmotic concentration as sea water and therefore impose a considerable salt load. It is no surprise, therefore, that in the petrel the salt concentration of the nasal fluid is higher than in any other

Table 3

Typical Composition of the Fluid from the Salt-Secreting Gland of Herring Gulls

Na^+	718 mEq./L.	Cl^-	720 mEq./L.
K^+	24 mEq./L.	HCO_3^-	13 mEq./L.*
$Ca^{++} + Mg^{++}$	2.0 mEq./L.	SO_4^{--}	0.68 mEq./L.

*Dr. Maren has kindly permitted me to quote average figures obtained in his laboratory. For nasal secretion these are: HCO_3^-=9.1 mEq./L., pH=7.03; and for plasma: HCO_3^-=21.7 mEq./L., pH=7.48.

Table 4

*Usual Concentration of Sodium in the Nasal Secretion of Different Species of Birds**

Species	Concentration of sodium, mEq./L.
Cormorant, double-crested	500-600
Duck, mallard	(550)
Skimmer, black	550-700
Eider duck, common	(625)
Pelican, brown	600-750
Gull, herring	600-800
Gull, black-backed	700-900
Penguin, Humboldt's	725-850
Guillemot	750-850
Albatross, blackfooted	800-900
Petrel, Leach's	900-1,100

*Some samples fall outside these limits, which represent only the usual range. Numbers in parentheses represent species in which samples have been too few to establish a normal range.

bird we have examined, up to 1,100 or 1,200 mEq./L.

No other gland in higher vertebrates, except the mammalian kidney, can produce fluids which are concentrated to this degree. The concentration limit for electrolytes in the kidney of man is about 400 mEq./L., in the rat 600 mEq./L., in the kangaroo rat, 1,500 mEq./L., and in the champion concentrator, the North African rodent *Psammomys*, 1,900 mEq./L. Thus, the salt gland compares favorably to the kidney in its concentrating ability. In other respects the salt gland is quite different from the kidney; it secretes only sodium chloride, it always produces a liquid which is highly hypertonic to the blood, and it works as an all-or-none system, becoming active only after an osmotic load.

Table 5

A Comparison Between the Rate of Secretion from the Salt Gland of the Gull and the Volume of Urine During Water Diuresis in Man

Flow per Kg. body weight:	Salt gland, herring gull	0.5 ml./min.
	Kidney, water diuresis, man	0.24 ml./min.
	Kidney, GFR, man	1.8 ml./min.
Flow per Gm. gland:	Salt gland, herring gull	0.6 ml./min.
	Kidney, water diuresis, man	0.03 ml./min.
	Kidney, GFR, man	0.2 ml./min.

Volume of Flow

Although the concentration of the nasal secretion is relatively constant, its volume is not. The rate of flow can change from zero to a maximum which varies with the species and, to some extent, between individuals within the species. After a salt load is given to a bird, the rate of flow rapidly increases, and remains rather high for a period that depends on the degree of the load. It then tapers off and diminishes as the salt is eliminated (table 2). However, the rate can remain at near-maximal levels for hours. That this rate is quite high becomes clear when the flow from the gland in the gull is compared wth the flow of urine during water diuresis in man. In table 5 flows are calculated per kilogram of body weight. It may be seen that although the osmotic concentration of the nasal secretion is high, the production of nasal fluid is more than twice the maximum water diuresis in man.

Size of the Gland and Flow Rate

What is the size of a gland which has this amazingly high excretory capacity? Its size is not very impressive compared to the kidney; in the gull, which has a relatively large salt gland, it is about 0.1 per cent of the body weight, while the kidney of mammals approaches about 1 per cent of the body weight. Thus, if we relate the volume of secretion to the weight of the gland rather than to body weight, the capacity of the salt gland becomes even more striking. The gland of the gull can produce about 0.5 ml. of fluid per Gm. of gland per minute. The glomerular filtration rate in man is about 0.2 ml. per Gm. of kidney per minute and in the rat, I believe, about 0.5 ml. per Gm. of kidney per minute. In other words, despite the considerable osmotic work necessary to produce the concentrated fluid, the salt gland can secrete at a rate which is as high as, or higher than, the glomerular filtration rate in the mammalian kidney.

Structure of the Gland

What is the structure of this unusual gland which produces fluid as fast as the kidney filters the plasma? Figure 1 shows the location of the gland in the gull. On top of the skull there are 2 crescent-shaped, flat glands, which are located in shallow depressions in the bone. Actually, each gland consists of 2 parts which are so similar in structure and are so closely joined that they can be considered as one functional unit, i.e., as one gland.[11] Two ducts run from each side down to the nose where they open at the vestibular concha. From the anterior nasal cavity the secretion flows out through the nares and drips off from the tip of the beak. A few marine birds have closed and nonfunctional external nares; characteristically these birds, e.g., gannets and cormorants, are good divers and enter the water after a fast dive from some height. The closed nares probably are a protection against the penetration of water at the time of impact with the surface. In these birds the nasal secretion flows through the internal nares along the roof of the mouth, forward to the tip of the beak.

The glands consist of longitudinal lobes which in cross section show tubular glands radiating from a central canal (fig. 2). Except for the most distal portion where the cells are somewhat smaller but of the same general

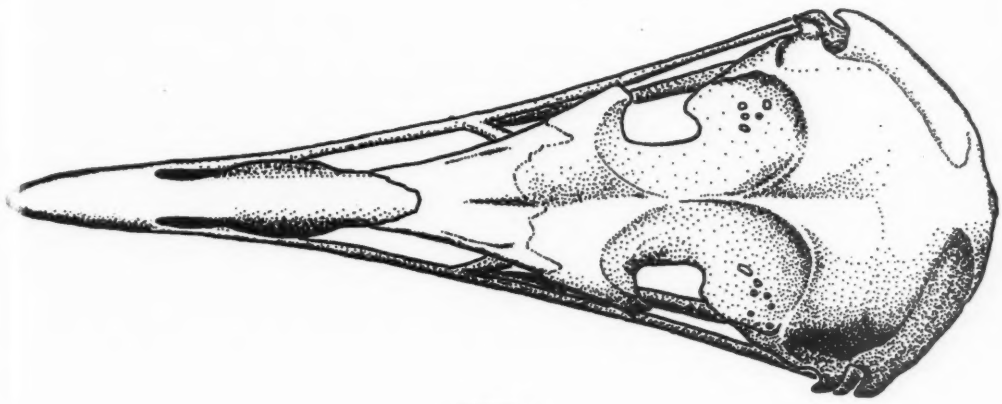

Figure 1

The skull of the herring gull shows the location of the salt-secreting glands as 2 shallow, crescent-shaped depressions in the bone above the orbit of the eye. (Drawing by M. Cerame-Vivas.)

type (fig. 3), the branching, tubular glands have a rather uniform structure throughout their length. The tubules are closed in the distal end, and there is no structure reminiscent of the glomerulus of the kidney. The structure is characteristic of a tubular gland, which indicates that the secreted fluid is elaborated only by simple glandular secretion, and that there is no ultrafiltration of fluid comparable to that in the kidney. This conclusion is supported by the fact that inulin does not appear in the secretion.

A retrograde injection of india ink into the gland through its duct shows that the ink penetrates all the way into the cells, giving the appearance of intracellular canaliculi which probably have a secretory function. Electron micrographs made by Dr. W. L. Doyle at the University of Chicago, as well as by other investigators, show that there are deep infoldings both in the apical and the basal parts of the secreting cell, which has some similarity to the tubular cell of the nephron. It must be assumed that the deep infoldings are intimately related to the function of the cells, and it could be suggested that the elaboration and transport of fluid does not necessarily take place as a transtubular process. If the infoldings prove to be similar to the interdigitations of the renal tubular cells as reconstructed by Rhodin, the transport process might indeed take place in the intercellular spaces of interdigitating cells, rather than across the cell body. These studies are being continued by Dr. Doyle and will be published elsewhere.

Blood Supply to the Gland

The gland receives its main arterial blood supply from the arteria ophthalmica interna. In the gull, several blood vessels penetrate the bony wall of the orbit and enter the gland from below. This makes the blood vessels difficult to reach, and attempts at perfusion of the gland are likely to meet with immense technical difficulties.

The circulation in the lobes of the gland can be visualized most easily in preparations which have been prepared by the injection of the vascular system with india ink.[11] It is found that the main arteries to the gland branch into interlobular arteries, which send smaller branches into the single lobes. These arteries continue toward the central canal, where they split up into capillaries which run parallel to the tubular glands toward the periphery of the lobe. A section cut perpendicular to the tubular glands (fig. 4) shows that the capillaries are interspersed between the glands, much as in the papilla of the kid-

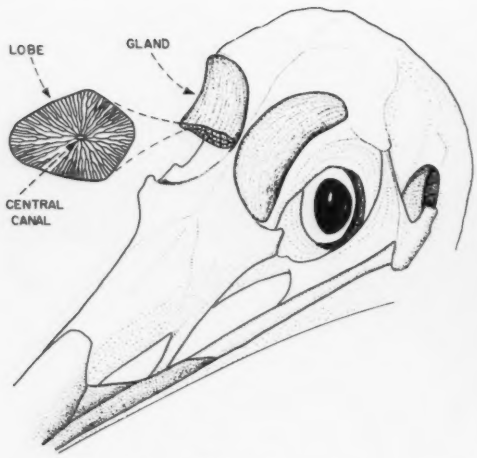

Figure 2

The salt gland of the gull consists of longitudinal lobes about 1 mm. in diameter; each lobe has a central canal with the branching secretory tubules arranged radially around it. (Drawing by M. Cerame-Vivas.)

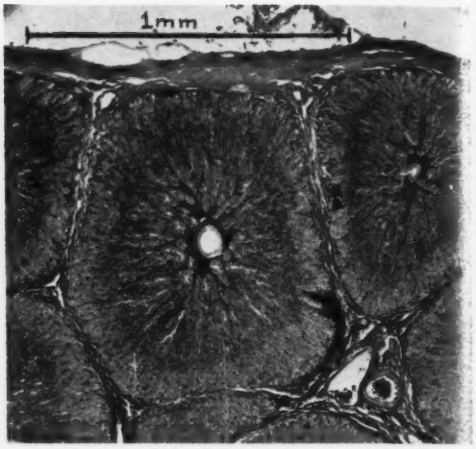

Figure 3A

In cross section the lobe of the salt-secreting gland shows the central canal and the radial arrangement of the secretory tubules. In the lower right hand corner an interlobulary vein and an artery are visible.

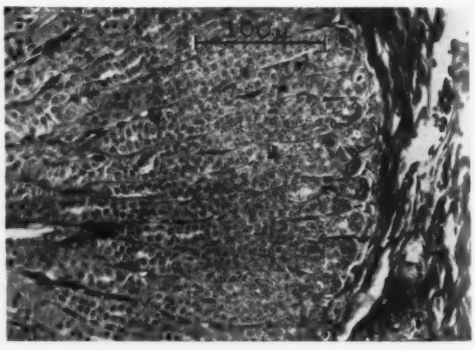

Figure 3B

In higher magnification the peripheral ends of the secretory tubules appear as closed tubes without any similarity to the glomerular apparatus of the kidney. However, the peripheral part of the tubule has smaller cells and an appearance which differs from the more central parts.

ney where the vasa recta are interspersed between the limbs of Henle's loop. A diagram of the circulation (fig. 5) shows how the blood flows parallel to, but in the opposite direction from, the flow in the tubular gland.

Is There a Countercurrent System in the Salt Gland?

The arrangement just described can, of course, be recognized as a countercurrent flow, and no renal physiologist is unaware of the importance of the countercurrent multiplier system in the elaboration of a concentrated urine in the kidney. However, in the case of the salt gland, the countercurrent flow cannot be used to explain the elaboration of a concentrated fluid. A countercurrent multiplier system consists of a tube which has been turned back on itself to form a loop; but in the salt gland we have 2 unconnected tubes running in opposite directions. This is typical for a type of countercurrent exchange system which is particularly well suited for obtaining the same concentration in the outgoing fluid from one tube as in the incoming fluid in the other (fig. 6). In other words, the system in the salt gland seems ideal for the elaboration of a secretion which is isotonic with plasma, and the high concentrations which are actually produced cannot result from a countercurrent multiplier system.

It is true that the direction of the blood flow may be opposite to what we have assumed for there is always some doubt about

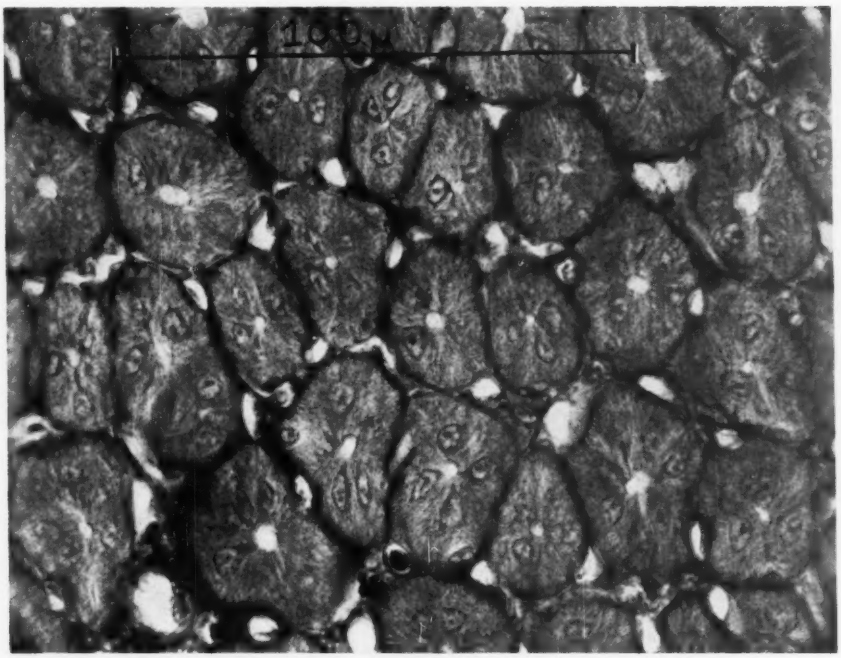

Figure 4

As may be seen in this cross section, the secreting tubules are interspersed with capillaries, much like the arrangement in the papilla of the kidney. Compare with the diagrammatic cross section to the right in figure 5.

the interpretation of histologic sections. But even if the blood flow were the reverse of what we have suggested, with the flow in the same direction as the fluid in the gland, the arrangement would still not be a multiplier system. We must therefore conclude that, although there is a countercurrent flow, the countercurrent multiplier principle cannot be used to explain the function of this particular gland and its ability to elaborate highly concentrated solutions.

Nervous Control

The nerve supply to the salt gland is rather complex and not easily worked out because the nerves in the head of the bird have a complex course with frequent anastomoses. The gland is innervated from a ganglion in the orbit, the ganglion ethmoidale. This ganglion is supplied by a relatively large branch of the ophthalmic nerve, and a smaller one from the facial nerve, as well as by sympathetic fibers. Apparently nonmyelinated fibers from the ganglion penetrate the bone to reach the gland. Electric stimulation of the ophthalmic nerve causes no secretion, but if the small branch from the facial nerve is stimulated, a fluid of the usual composition, rich in sodium chloride is secreted. As far as we can establish, the branch of the facial nerve continues back toward the inner ear; it is reminiscent of, and could be homologous to, the chorda tympani which stimulates salivary secretion in mammals.

The secretory nerve apparently is parasympathetic in nature.[12] Stimulation of the cervical sympathetic chain causes no secretion and injection of epinephrine blocks secretion from the gland. Acetylcholine, if injected in a proximal artery, causes the gland to secrete; if injected in the general circulation it will, of

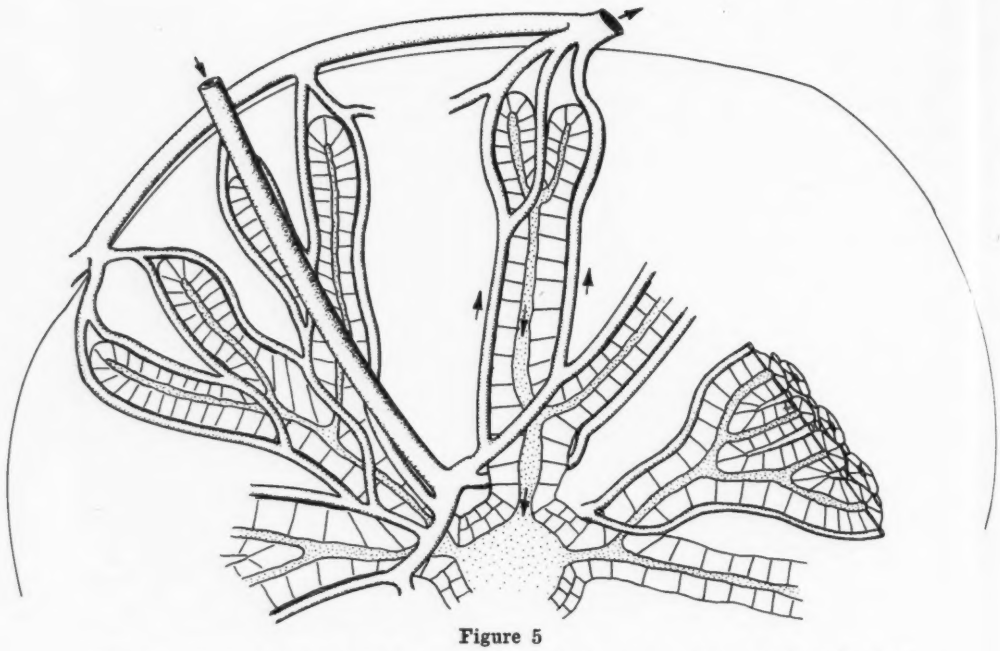

Figure 5

The arrangement of the blood vessels and capillaries in the lobes of the salt gland indicates that the capillary blood flow is countercurrent to the flow of secreted fluid in the secretory tubule. (Drawing by M. Cerame-Vivas.)

course, be hydrolyzed before it reaches the gland. The parasympathomimetic substance methacholine (Mecholyl) also causes the gland to secrete.

Effect of Anesthesia

One difficulty in the study of the function of the salt gland is that the secretion is blocked by anesthesia. Secretion can always be induced in the nonanesthetized bird by an osmotic load, for example by the injection of hypertonic sodium chloride. Within a few minutes, sometimes within 1 minute of an injected load, secretion will begin. However, since anesthesia immediately blocks secretion, many further experiments, in particular those involving surgical procedures, cannot be carried out.

Osmoreceptor Reflex

The effects of anesthesia, of nerve stimulation, and of osmotic load, indicate a sequence of control as suggested in figure 7. The term osmoreceptor is used, rather than salt receptor, because the gland responds not only to salt loads, but to osmotic loads in general. If a hypertonic solution of a nonelectrolyte such as sucrose is infused, the gland begins to secrete a fluid of the usual composition, with a high concentration of sodium chloride. This indicates that the secretion occurs in response to an osmotic load, rather than to the concentration of sodium or chloride in the plasma.

Carbonic Anhydrase in the Salt Gland

It has frequently been proposed that carbonic anhydrase plays an important role in ionic transport mechanisms. Since the salt gland is an organ which apparently has no other function than the transportation of sodium chloride, it seems well suited to a study of the possible role of this enzyme in the mechanism of transport. Many schemes which have been suggested to account for ionic transport involve bicarbonate and car-

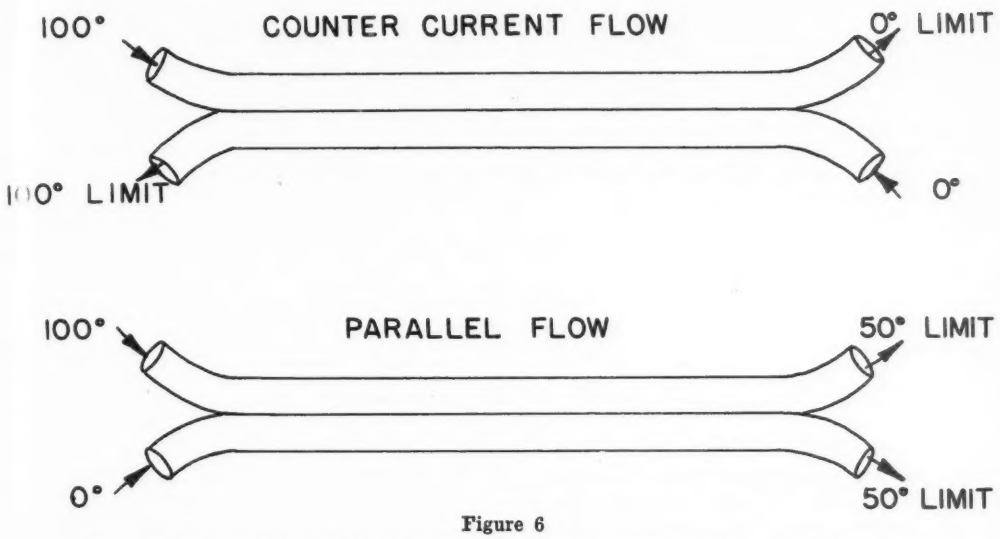

Figure 6

Top. *Thermal models indicate how the heat flow in a countercurrent exchange system leads to equal temperature in the affluent from one tube and the effluent from the other.* Bottom. *A parallel flow leads to equal temperature in the effluent from both tubes. These models assume equal volumes of flow in the 2 tubes. If the flows differ the limits change accordingly, but in no case can a passive exchange system increase the temperature in the effluent from one tube to a level above that in the other.*

bonic anhydrase in an ion exchange system. With Dr. T. H. Maren, in 1958, we found that the salt gland is rich in carbonic anhydrase.

Of considerable interest is the observation that the carbonic anhydrase inhibitor, acetazoleamide (Diamox), when injected into a bird whose gland is actively secreting, blocks the secretion almost instantaneously.[12] This result indicates that carbonic anhydrase is essential to secretion, but it does not prove that the block is in the ionic pump of the secreting cells. A possible explanation is that the block could be somewhere else in the pathway indicated in figure 7. To examine this possibility experiments were done to determine if the gland retains its ability to secrete in the presence of the enzyme inhibitor. This has actually been found to be the case: direct stimulation of the gland with methacholine gives a secretion of the usual composition, showing that the ionic pump can work in the presence of a carbonic anhydrase inhibitor. Another possible interpretation, however, is that the inhibition of the secreting cells is a matter of relative concentrations, and that, if the stimulus is strong enough, the gland starts secreting in spite of the presence of the inhibitor. Further work on this point has been done by Dr. James Larimer, a former student of mine, in Dr. Maren's laboratory.

The Role of Acetylcholine in Secretion

It is possible that the parasympathetic nature of the gland affords a clue to the mechanism of ion transport. The secretion occurs in response to the release of acetylcholine from the stimulating nerve. This pattern is reminiscent of other systems in which acetylcholine has an important influence on the movement of sodium, such as in the depolarization of the muscle fiber and of the nerve fiber. Recently, the Hokins of the University of Wisconsin have demonstrated that slices of the salt gland in vitro, when incubated with acetylcholine, show a greatly increased turnover of phosphatidic acid, as evidenced by radiophos-

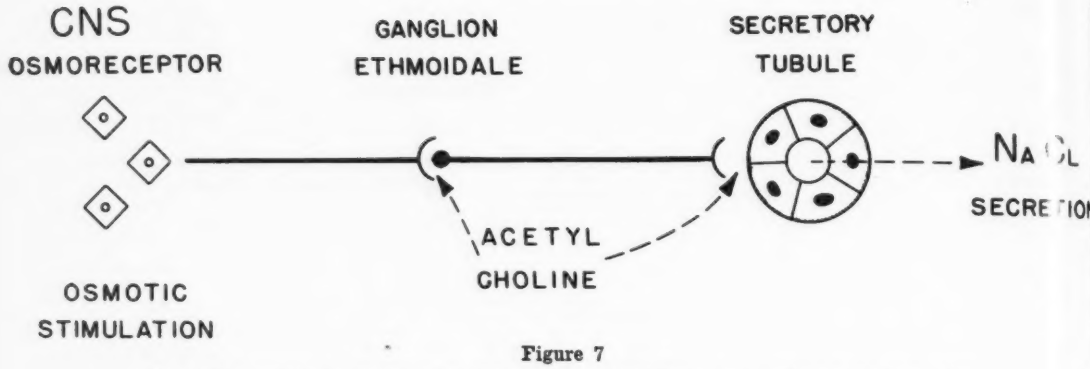

Figure 7

Suggested sequence of events in the osmotic stimulation of secretion from the avian salt gland.

phorus tracer experiments.[13] The Hokins suggest that phosphatidic acid is an integral part of the sodium pump in the gland, and that it may be the actual ionic transport agent itself.

In a recent series of papers[14–16] Scothorne has described histologic and histochemical studies of the nasal gland of ducks and pigeons. The gland of the domestic duck, like that of the mallard, its wild ancestor, does secrete salt, but the gland of the pigeon does not. In the gland of the duck there is a moderate amount of alkaline phosphatase, and a high content of succinic dehydrogenase. Not all glands have a high content of succinic dehydrogenase; it seems probable that the presence of this enzyme in the salt gland may be closely related to the energy which is required to perform an exceptionally high level of osmotic work.

Obviously, much work remains to be done on this unique gland, which apparently has no other function than that of transporting sodium chloride and therefore seems to be eminently suited for studies of processes involved in ionic transport. I believe that it is too early to say whether the transport is due to a sodium pump or to a chloride pump. My personal guess is that it may be a chloride pump, but I have no real support for this notion except that the proportion of sodium to potassium in the secreted fluid may indicate that the cation follows passively. In either case, I expect that future work will answer the question. At this time I can only say that if there is any organ in which an ionic pump and its metabolic ramifications can be studied in its pure form, the salt gland of marine birds almost seems to be designed for this purpose.

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Water as a Substrate for Life

Water, of its very nature, as it occurs automatically in the process of cosmic evolution, is fit, with a fitness no less marvelous and varied than that fitness of the organism which has been won by the process of adaptation in the course of organic evolution.

If doubts remain, let a search be made for any other substance which, however slightly, can claim to rival water as the milieu of simple organisms, as the milieu intérieur of all living things, or in any other of the countless physiological functions which it performs either automatically or as a result of adaptation.—L. J. Henderson. *The Fitness of the Environment*. Boston, Beacon Press, 1958, pp. 131-132.

Ion Movement in the Eye

By V. Everett Kinsey, Ph.D.

The optical integrity and the metabolism of the eye depend on the exchange of substances between the blood vessels of the eye and the ocular chambers. The present paper considers the exchange of 3 ions in particular, i.e., sodium, chloride and bicarbonate, in the eye, with particular reference to the role of diffusion and active transport in determining the ionic composition of the various ocular fluids.

THE TRANSPORT of substances from the blood vessels of the eye into the ocular chambers maintains the pressure necessary for optical integrity and provides for the metabolic requirements of the avascular structures within. An increase in volume of fluid transported with a constant resistance to outflow, or an increase in resistance to outflow with a constant rate of flow raises the intraocular pressure and may result in glaucoma. Conversely, a decrease in the volume of fluid entering the eye against a constant resistance, or a reduction in the resistance to outflow at constant volume of flow results in ocular hypotension.

Constituents of the plasma move into and out of these chambers across bounding membranes which offer degrees of resistance which vary from substance to substance and from place to place within the eye. Once within the ocular chambers, movement by flow is nondiscriminatory and that by diffusion is dependent only on molecular mobility. The main regions of transport from the blood to the intraocular chambers are: (1) the epithelium of the ciliary processes (fig. 1); (2) the capillary walls and epithelium of the iris (fig. 2); and (3) some of the retinal layers such as the external limiting membrane and Bruch's membrane (fig. 3).

In part, because of the varying rates of passage of substances through the barriers lying between the blood and the aqueous and vitreous humors, their compositions vary from each other and from that of the blood (table 1).[1]

There is bulk flow of fluid from the ciliary processes into the posterior chamber and out through the lens-iris space into and through the anterior chamber where it leaves the eye through the trabecular meshwork at the angle formed by the iris and cornea.

Movement occurs also by diffusion within the posterior and anterior chambers, between the posterior chamber and the lens and vitreous humor, and between the anterior chamber and the lens and cornea. Also, there is probably an active exchange into and out of both the cornea[2] and the lens.[3] The structures involved are shown in the cross-sectional view of the anterior segment of a human eye (fig. 4).

I will stress chiefly the movement of the 3 physiologically important ions, Na, Cl, and HCO_3, from the blood into and between the posterior chamber, anterior chamber, and the vitreous body of rabbits. Quantitative determination will be made of the proportion of each ion which enters the different parts of the eye by active secretion and by diffusion; the ionic composition of the secreted fluid will be estimated from the coefficients so determined. The quantitative approach employed applies to the study of the transport of other ions and nonelectrolytes into the ocular chambers, and to movement in and out of the lens and cornea. Space limitations preclude a detailed description of movement in these latter structures and an account of the effect of

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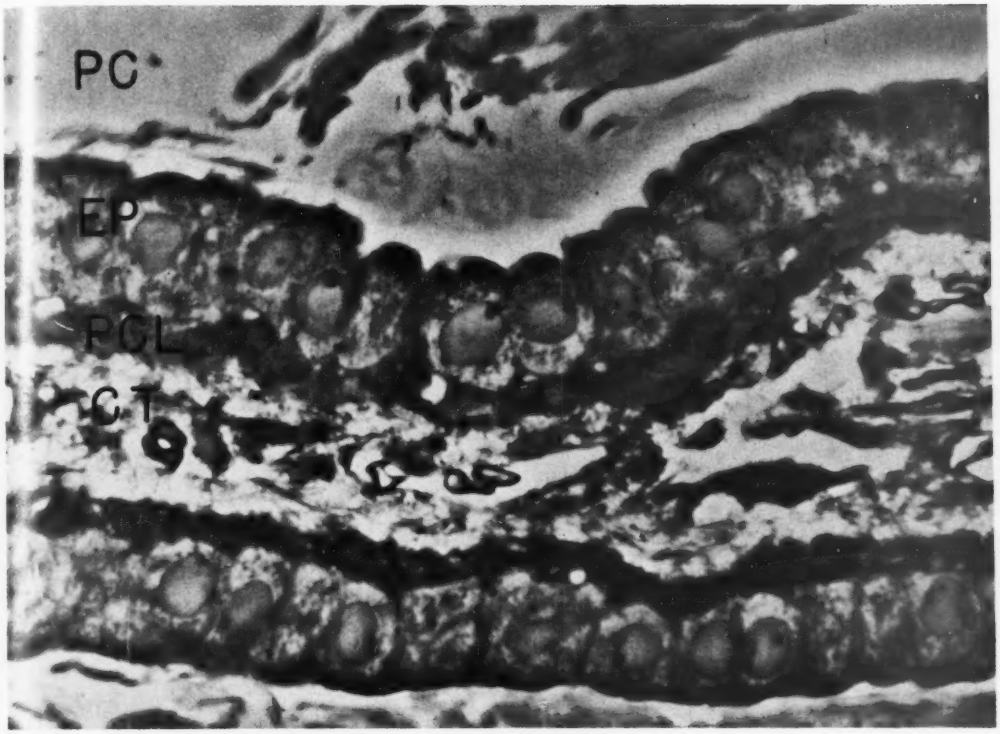

Figure 1

Ciliary process of albino rabbit. PC: *posterior chamber.* EP: *epithelial cell layer.* PCL: *pigment cell layer.* CT: *connective tissue containing many blood vessels. Phase micrograph by George D. Pappas, Columbia University.*

altering 1 of the parameters (flow) of the intraocular fluid system by administration of acetazoleamide (Diamox). These aspects of ion movement in the eye have been studied by a number of investigators.[2-7]

The mechanism of formation of the fluid in the posterior chamber has been the subject of speculation. A decade ago the author[8] proposed a unified concept of aqueous humor dynamics, the so-called "secretion-diffusion theory." It was suggested that the movement of constituents of plasma into the posterior chamber involved an electron transport or redox pump which resulted in the production of unbalanced hydroxyl ions and the simultaneous diffusion of all constituents of plasma into and out of the posterior chamber. The hydroxyl ions, through reaction with carbon dioxide, were thought to be converted to bicarbonate ions which were electrically neutralized by the entrance of Na and other cations from the blood. The salts which were formed in this way diffused into the posterior chamber, forming an aqueous humor hypertonic to the plasma. Because of the hypertonicity, more water was believed to diffuse into the posterior chamber than diffused out again, thereby resulting in net transport of water into the posterior chamber.

The observation, some 3 years later,[9] that the concentration of HCO_3 in the aqueous humor of the posterior chamber is even higher than that in the aqueous humor of the anterior chamber, seemed to lend support to the idea that bicarbonate formation may be involved directly in the production of aqueous

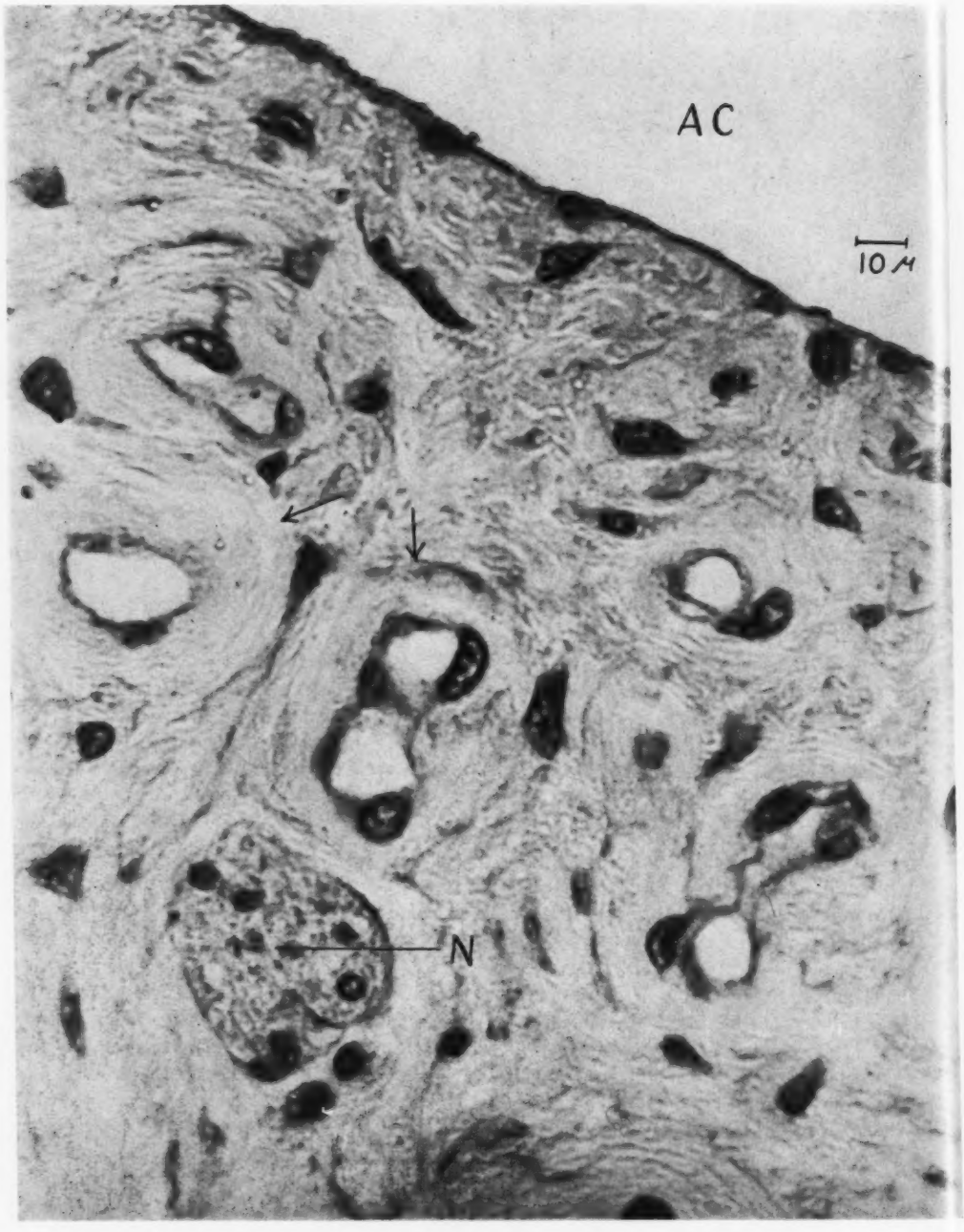

Figure 2

Anterior surface of the iris of the albino rabbit. AC: *anterior chamber.* N: *nerve. Several large capillaries are shown. Concentric layers of collagen fibers about these small vessels are indicated at the arrows. Photomicrograph by George Smelser, Columbia University.*

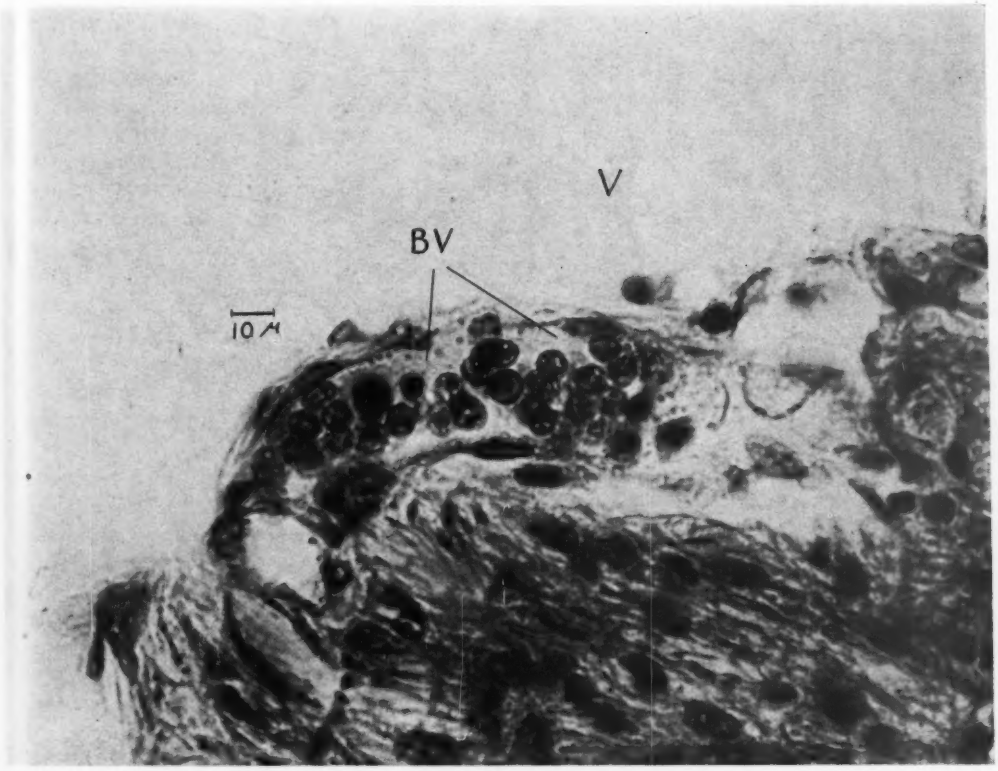

Figure 3

Retinal vessels in the albino rabbit. BV: *venule.* V: *vitreous humor. In the rabbit retina, particularly near the optic nerve, the blood vessels are extremely superficial, as shown here. Photomicrograph by George Smelser, Columbia University.*

humor. Recent evidence, however, shows that the oxygen consumption of the ciliary processes is insufficient to produce the required number of hydroxyl ions[10] to account for the entrance of all of the sodium into the posterior chamber. Moreover, Auricchio and Bárány[11] have shown that the mobility of water is far too low to enable the osmotic gradient between the aqueous and the plasma to move the amount of fluid which flows from the plasma to the aqueous. They also question the existence of such an osmotic gradient. Experiments by Auricchio and Wistrand[12] have demonstrated, too, that acetazoleamide in doses which cause considerable reduction in net flow has no effect on the osmotic gradient between the aqueous and the plasma. Levene[13] also questions the physiologic importance of a hypertonic aqueous in transporting fluid into the eye.

Thus, doubt has been cast on the idea that the bulk flow of aqueous can be accounted for by the entrance of Na into the posterior chamber as an indirect result of the action of a redox pump followed by the osmotic entrance of water. Alternative mechanisms to account for the active transport of Na, not only into the eye but across membranes in general, will probably await information gained from the study of other membrane systems which are more accessible to study. Nevertheless, in accounting for the movement of ions into and out of the posterior chamber from the blood, and for the bulk flow of fluid through the

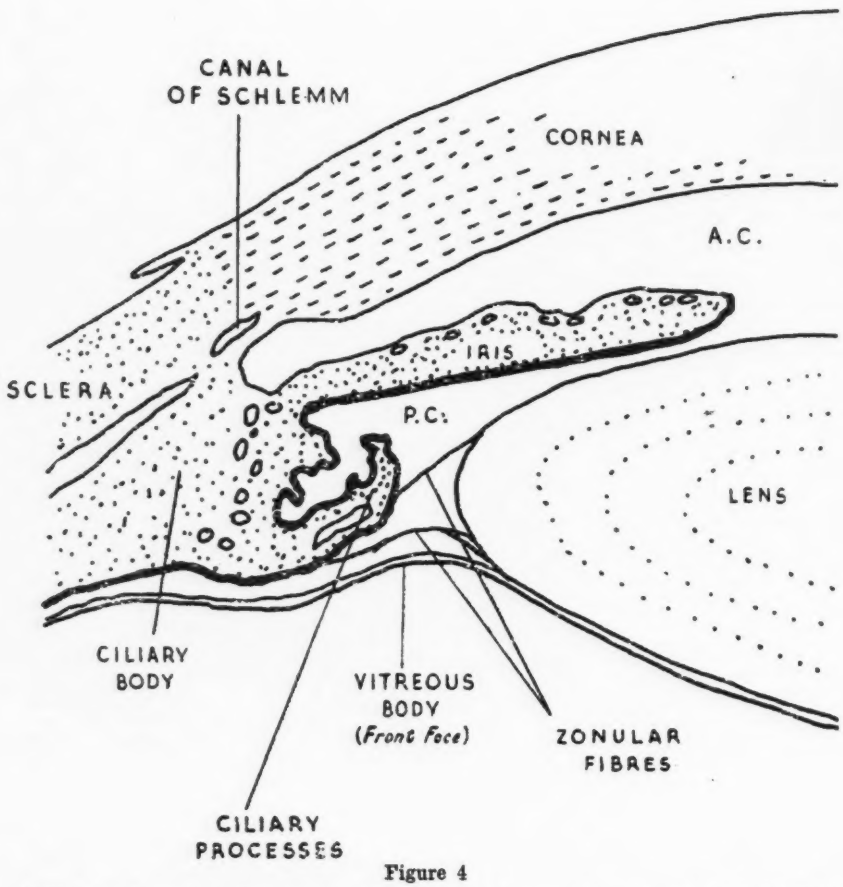

Figure 4

Anterior segment of a human eye. (Republished by permission of Blackwell Scientific Publications.[30])

eye, it is hoped that the following considerations will do more than push our limits of ignorance from the posterior chamber into the infoldings of the cell membranes of the ciliary epithelium!

A hypothesis consonant with the studies referred to above is that fluid is formed in the epithelium of the ciliary processes, rather than in the posterior chamber, and that it is this fluid which flows into the posterior chamber as a result of a "secretory pressure" or, alternately, emerges as a "package," e.g., by pinocytosis. The entrance, if not indeed the formation, of such a fluid in the ciliary processes, would require the expenditure of energy, and its unidirectional transport may be defined as secretion. No a priori assumptions need be made concerning the exact location of a secreted fluid before it enters the posterior chamber, but conceivably, it may be contained in the vesicles shown by Holmberg,[14] and more recently by Pappas and Smelser,[15] to be present in the ciliary epithelium.

Because all biologic membranes are more or less "leaky," substances which are transported across the ciliary epithelium unidirectionally may also move bidirectionally, i.e., enter and leave by diffusion. That a dual mechanism of entrance is involved in the case of at least 1 ion is suggested from the fo

lowing considerations. The steady state concentration of ascorbic acid in the aqueous humor of the posterior chamber is some 25 times the concentration of ascorbic acid in plasma.[16] In the absence of evidence that this acid is produced intraocularly,[17] the large difference in concentration suggests the existence of 1 or more unidirectional transport mechanisms, and it is generally agreed that ascorbic acid is secreted into the posterior chamber.

Some years ago I showed[16] that the concentration of ascorbic acid in the aqueous humor rises linearly with levels in the plasma up to about 3 mg. per cent. Above this concentration the secretory mechanism apparently becomes saturated. When the concentration in the plasma is raised to very high levels, i.e., above those which can be maintained in the aqueous humor by the secretory mechanism, Friedenwald[18] observed that a further increase in the concentration in the posterior aqueous occurs; presumably, this increase in the concentration of ascorbic acid is brought about by a bidirectional transport mechanism, i.e., diffusion. Thus, there is evidence for both a uni- and a bidirectional transport of 1 substance across the blood-aqueous barrier of the ciliary process.

Figure 5 shows the situation diagrammatically. Substances may enter the posterior chamber from the blood across the ciliary epithelium by diffusion or flow. Those which enter by diffusion obviously leave, in part, by the same route, indicated by lines having double arrows. Those which enter by flow (secretion), however, must leave in part, at least, by some other means, or by another route, since flow is unidirectional—indicated by single arrows. Diffusional exchange between posterior chamber and vitreous humor is also shown in figure 5 by lines with double arrows; movement by flow to the anterior chamber is shown by the vertical line with a single arrow.

Through an analysis of the rates of accumulation of the isotopes Na^{24} and Cl^{36} in the posterior chamber, Reddy and I[19] have analyzed the mode of entrance of ions from the blood to the posterior chamber, i.e., the proportion of ions transported by diffusion and flow. We also have made an estimate of the ionic composition of the secreted fluid as it emerges from the cells of the ciliary processes into the posterior chamber.

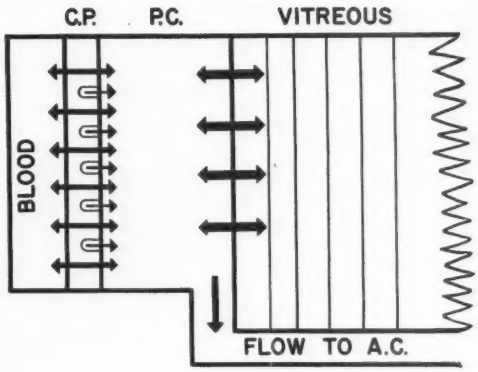

Figure 5

*Movement of substances into and out of the posterior chamber shown diagrammatically (*CP: *ciliary process,* PC: *posterior chamber,* AC: *anterior chamber).*

With respect to the anterior chamber, the proportions of the Na and Cl which enter as a result of flow from the posterior chamber and by diffusion across the iris vessels have also been calculated.

Posterior and Anterior Chambers

The major factors which control the accumulation of a substance in the posterior chamber are:

1. The concentration in the secreted fluid.
2. The rate of flow of the secreted fluid into the posterior chamber.
3. The rate of flow of the aqueous humor from the posterior chamber to the anterior chamber.
4. The rate of diffusion between blood and posterior chamber.
5. The rate of diffusion between posterior chamber and vitreous humor and lens.

The proportion of the total amount of any substance entering the posterior chamber by diffusion and flow can be approximated from

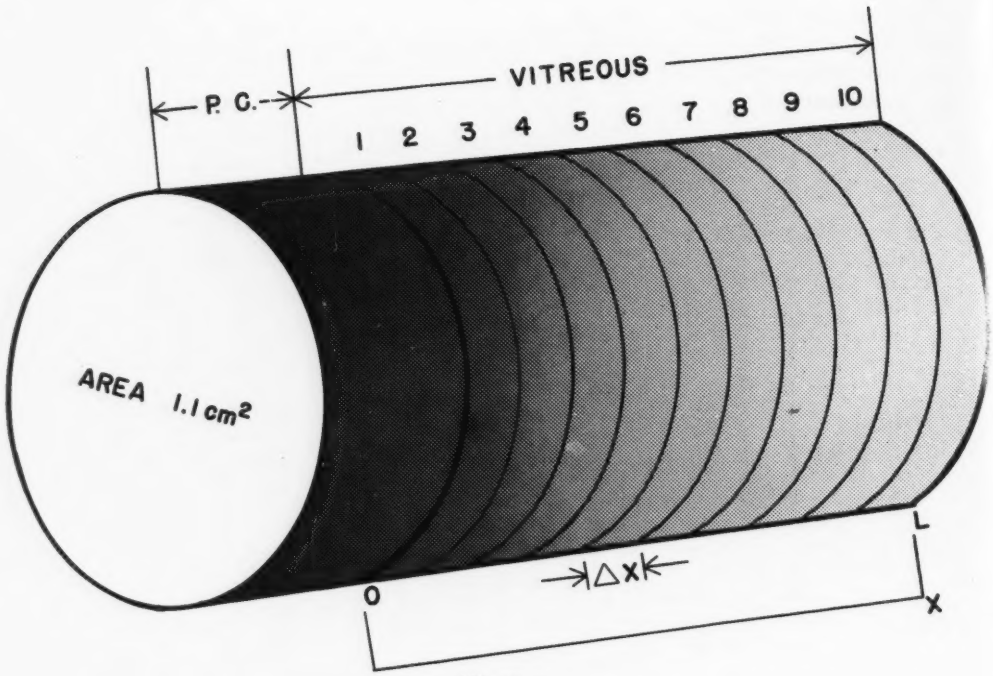

Figure 6

Model of posterior chamber and vitreous considered as a cylinder with 10 equally spaced stations or compartments in which the cross-sectional area is equal to that of the posterior chamber–vitreous interface. The depth of shading illustrates a concentration gradient at a particular time as a function of the distance away from the posterior chamber.

an analysis of the time course of entrance of the substance from the blood, and the ratio of concentrations in aqueous and plasma at steady state, if the rate of loss by flow to the anterior chamber and by diffusion to the lens and vitreous is known.

One of the difficulties in the mathematical analysis of experimental data involving the transfer of substances into the posterior chamber arises from the fact that the concentration of a test substance in the blood (e.g., an isotope) is seldom constant throughout the time of observation. A more serious complicating factor stems from the difficulty of estimating the loss of the test substance to the vitreous and lens.*

*About one-tenth as much sodium and chloride is lost to the lens as to the vitreous humor, and since the mathematical model to be described is only an approximation of the geometry of the eye, I will refer only to the loss to the vitreous hereafter.

Palm and I[20] employed the method of numerical integration to analyze results obtained with radioactive Na. This method took into account the changing concentration in the plasma but made no allowance for the fact that the vitreous humor is not well mixed. Friedenwald and Becker[21] devised a more elaborate mathematical method of treating the same data which involved exponential analysis; it allowed for neither a varying concentration of plasma nor for the lack of mixing in the vitreous. A graphic method of integration has been used by Maurice[22] which compensated both for the varying plasma concentration and for the loss to the vitreous; but, it did not distinguish between the amount entering the posterior chamber by secretion and the amount entering this chamber by diffusion.

Recently Dr. Reddy and I[19] have analyzed data on the accumulation of both Na and C

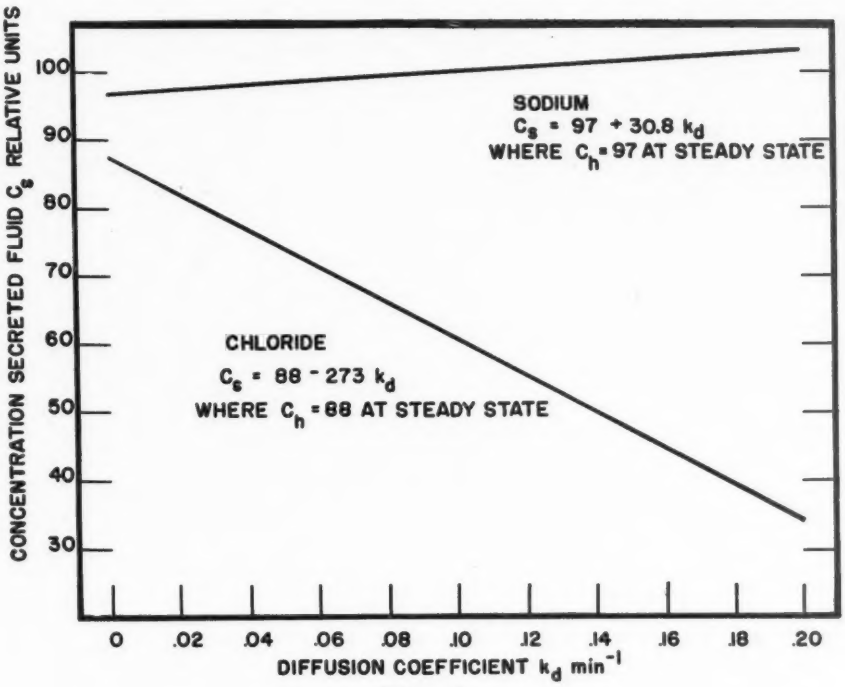

Figure 7

The relation between the concentration of sodium and chloride in the secreted fluid and the coefficient of diffusion between the plasma and the posterior chamber, assuming the concentrations at steady state in the aqueous of the posterior chamber and vitreous humor are equal.

by a method which takes into account varying concentrations in the plasma and loss to the vitreous without making the restrictive assumption that this chamber is well mixed. This was accomplished by the use of an electrical analog computer. The difficulty arising from the changing concentrations of these substances in blood was overcome by feeding values of the actual concentration of the substance in the blood into the computer as an input; the difficulty associated with loss to the vitreous was overcome by treating the concentration in the vitreous as a function of time and position. This more general method of treatment leads to a partial differential equation which relates time, concentration, and distance traversed by the test substance into the vitreous. It also makes it possible to distinguish between entrance by flow and diffusion.

In the treatment of the analytic data, a further refinement which introduced no mathematical complications was the introduction of a term α in the equation describing entrance by diffusion into either the posterior or the anterior chamber. This refinement compensates for differences in the electrochemical activity of anions and cations developed as a result of the presence of protein in the plasma (Donnan effect). The numerical values used for α were those given by the inverse relation between the concentrations at steady state as predicted by the Gibbs-Donnan effect, i.e., 1.05 and 0.95 for Na and Cl, respectively, viewed from the side of the intraocular chamber. These values are closely similar to the theoretic ones calculated by Van Slyke (0.96 and 1.04).[23]

No attempt was made to take into account other possible electrochemical potential differ-

Table 1

*Concentration of Various Substances in the Intraocular Fluids of the Eye of the Rabbit**

Sample	Sodium	Potassium	Chloride	Total CO_2	Phosphate	Ascorbate	Lactate	Glucose	NPN
Vitreous	134	9.5	104.7	26.0	0.40	0.46	12.0	3.0	17
Posterior aqueous	136†	—	96.5	37.5	0.58	1.38	9.9	5.6	23
Anterior aqueous	138	5.0	101.0	30.2	0.89	1.11	9.3	5.4	25
Plasma	143	5.6	108.8	20.6	2.04	0.04	10.3	5.7	34

*All values are expressed in mM per Kg. of water.
†Estimated.

Table 2

Symbol	Designation	Units
A_{hv}	Area of posterior chamber–vitreous interface	$cm.^2$
α	Donnan factor	Dimensionless
C_a	Concentration in aqueous humor of anterior chamber	Relative units* or $\tilde{m}M/Kg.\ H_2O$
C_h	Concentration in aqueous humor of posterior chamber	Relative units or $mM/Kg.\ H_2O$
C_p	Concentration in plasma	Relative units or $mM/Kg.\ H_2O$
C_s	Concentration of secreted fluid	Relative units or $mM/Kg.\ H_2O$
C_v	Concentration in vitreous humor	Relative units or $mM/Kg.\ H_2O$
D	Diffusion constant	$cm.^2/min.$
$k_{d.hv}$	Transfer coefficient by diffusion posterior chamber to vitreous	$min.^{-1}$
$k_{d.ph}$	Transfer coefficient by diffusion plasma to posterior chamber	$min.^{-1}$
k_{fa}	Transfer coefficient by flow into and out of anterior chamber	$min.^{-1}$
k_{fh}	Transfer coefficient by flow into and out of posterior chamber	$min.^{-1}$
V_a	Volume of anterior chamber	$cm.^3$
V_h	Volume of posterior chamber	$cm.^3$
V_v	Volume of vitreous	$cm.^3$
x	Space variable	cm.

*A relative unit is defined as a percentage of the concentration in the plasma once the concentration reached in the plasma is essentially constant.

ences such as that known to exist across frog skin, since at the time this study was made no one had demonstrated an appreciable potential difference within the ciliary processes. Meanwhile, Berggren[24] has shown that the ciliary epithelial cells are about 70 mV negative with respect to the posterior chamber, but since there is little potential difference (5 mV) across the epithelium as a whole, i.e., between the stroma and the posterior chamber, the observed potential difference from the inside to the outside of the cells may have no special significance as far as transporting ions into the posterior chamber is concerned.

The rate of accumulation in the posterior chamber of a test substance which enters both by secretion and diffusion, and the loss to the anterior chamber by flow and to the vitreous and lens by diffusion, have been described previously[21] by an equation essentially identical to the following:

$$\frac{dC_h}{dt} = k_{fh}(C_s - C_h) + k_{d.ph}(C_p - C_h) - k_{d.hv}(C_h - C_v). \quad (1)$$

The meaning of the symbols in equation (1) and those equations that follow is given in table 2.

The last term of the equation adequately describes the situation only if the fluid in the vitreous humor is well mixed; moreover, the equation does not contain a term to describe the effect of the Donnan forces.

For the purpose of estimating the diffusional loss of a test substance through the unmixed vitreous, this body was considered to be a cylinder, having the same volume as

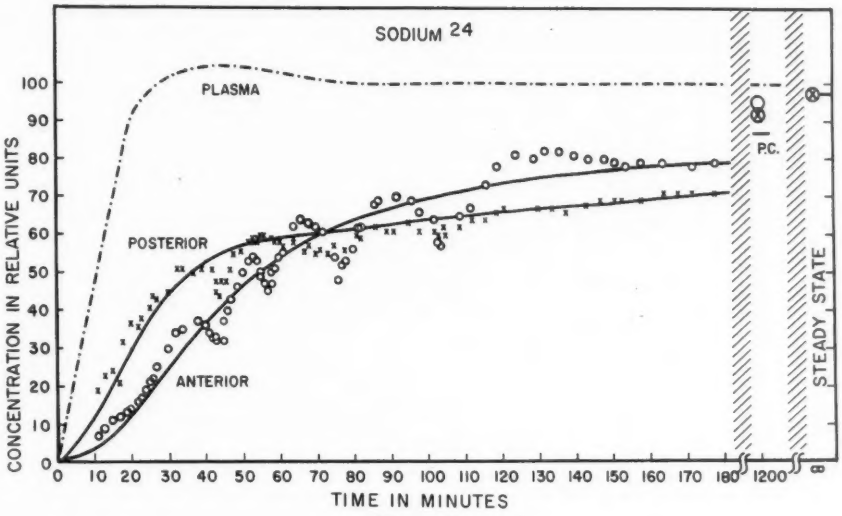
SODIUM 24
PLASMA
POSTERIOR
ANTERIOR
P.C.
STEADY STATE
CONCENTRATION IN RELATIVE UNITS
TIME IN MINUTES
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 1200 ∞

Figure 8

The concentration of Na^{24} in the plasma, posterior and anterior chambers of the rabbit eye at various times after parenteral administration. (Republished by permission of Documenta Ophthalmologica.[19])

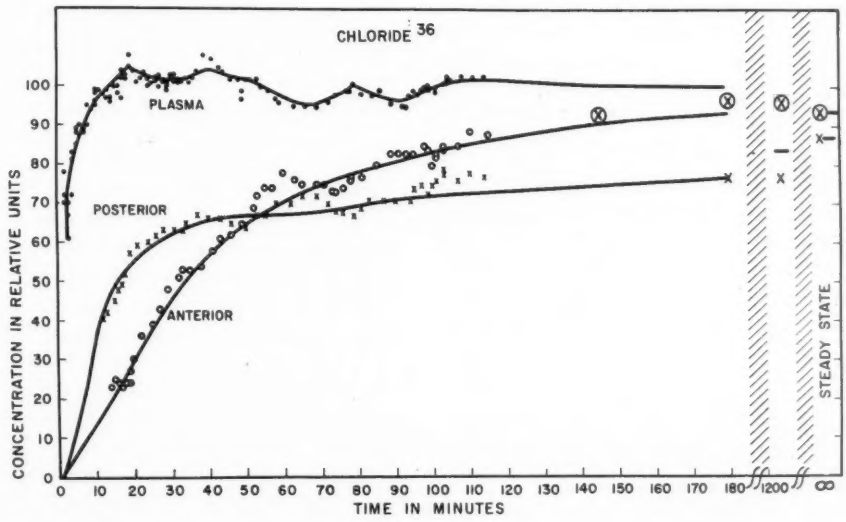
CHLORIDE 36
PLASMA
POSTERIOR
ANTERIOR
STEADY STATE
CONCENTRATION IN RELATIVE UNITS
TIME IN MINUTES
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 1200 ∞

Figure 9

The concentration of Cl^{36} in the plasma, posterior and anterior chambers at various times after parenteral administration. (Republished by permission of Documenta Ophthalmologica.[19])

he vitreous, and with a cross-sectional area equal to that of the interface between the posterior chamber and the vitreous. As illustrated n figure 6, discrete stations were chosen at equally spaced points throughout the vitreous.

The space derivative $\frac{\delta C_v}{\delta x^2}$ was approximated by finite differences so that an ordinary differential equation, with time as the independent variable, could be established at each of

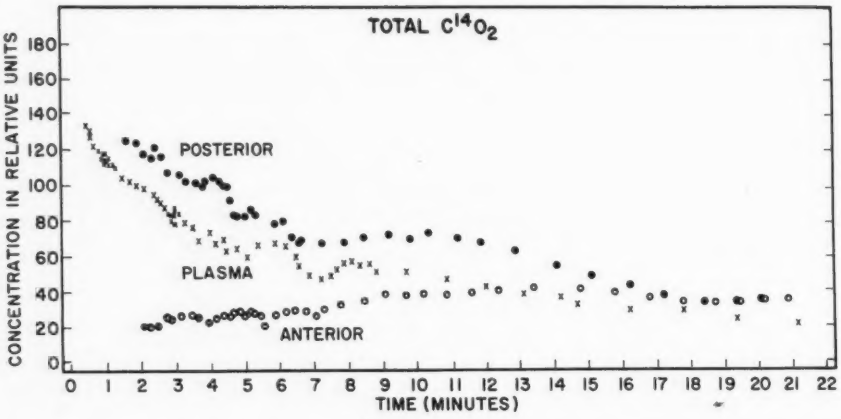

Figure 10

The concentration of C^{14}-labeled total carbon dioxide in the plasma, posterior and anterior chambers of the rabbit eye at various times after intravenous administration of C^{14}-labeled bicarbonate. (Republished by permission of A.M.A. Archives of Ophthalmology.[28])

the 10 stations in the vitreous. The resulting system of simultaneous differential equations for the vitreous, in conjunction with an equation to follow describing the accumulation of the test substance in the posterior chamber, was solved with the analog computer. The exchange between the posterior chamber and the vitreous was established from the approximation of the derivative so generated at the interface between the posterior chamber and vitreous, i.e., the first station only. The numerical values obtained were continually substituted for the last term of equation (2) and at any moment in time the value is equal to:

$$\left(\frac{DA_{hv}}{\Delta x \, V_h} \, (C_h - C_v) \right) \quad x = 0.$$

$x = 0$ is boundary between posterior chamber and vitreous.

Equation (2) describes the rate of accumulation of a test substance in the posterior chamber and differs from equation (1) in that it includes the vitreous term involving the space derivatives just discussed and the term α (Donnan factor).

$$\frac{dC_h}{dt} = k_{fh} (C_s - C_h) + k_{d,ph} (C_p - \alpha C_h) - \left(\frac{DA_{hv}}{\Delta x \, V_h} \, (C_h - C_v) \right) x = 0. \qquad (2)$$

Expressed in words, the equation says that the rate of change of concentration of any substance in the posterior chamber is equal to the flow rate times the difference in concentration between the "secreted" fluid and the fluid in the posterior chamber, plus the diffusion rate times the difference in concentration in the plasma water and the effective concentration in the posterior aqueous, minus the decrease in concentration owing to diffusional loss to the vitreous humor. The latter, in turn, is equal to a factor composed of the diffusion rate of the substance within the vitreous times the area of the posterior chamber–vitreous interface divided by the thickness of vitreous in the first station, times the volume of the posterior chamber, which is multiplied by the difference in concentration in the posterior chamber and vitreous.

The values for the concentration in the posterior chamber (C_h) and plasma (C_p) are known from experimental observations; that for the vitreous (C_v) is obtained as described above by computation. The flow rate (k_{fh}) in the rabbit eye is known from independent experimental data (perfusion,[25] tonography,[2] turnover of para-aminohippuric acid[26]) to be about 3.5 μl. per minute, which is equal to about 6 per cent of the volume of the posterior chamber per minute. The value for α depends upon the charge of the ion under considera-

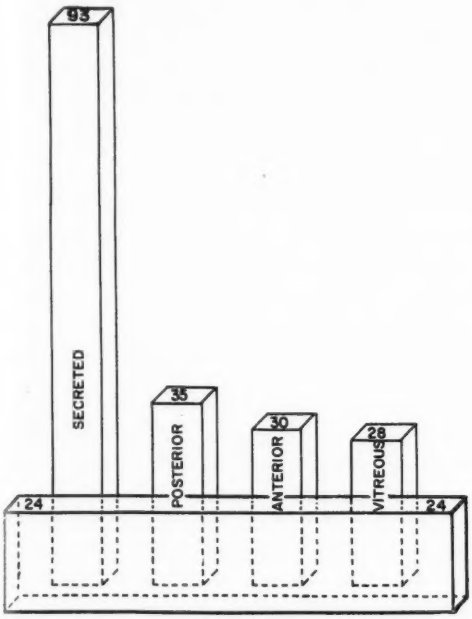

Figure 11

Relative concentrations of bicarbonate in the humors of the rabbit eye. (Republished by permission of Documenta Ophthalmologica.[19])

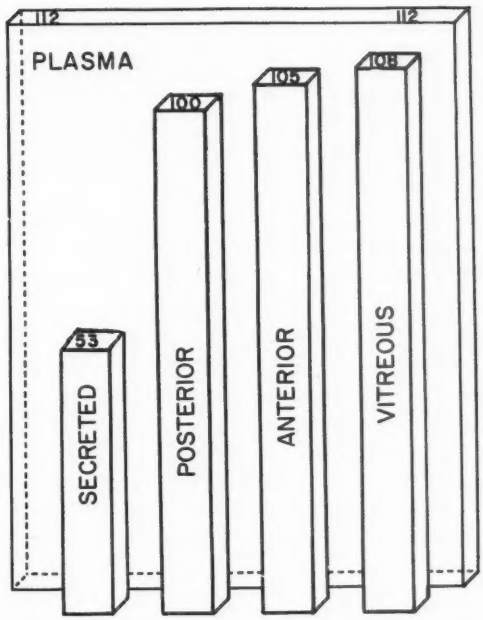

Figure 12

Relative concentrations of chloride in the humors of the rabbit eye. (Republished by permission of Documenta Ophthalmologica.[19])

tion and has been assumed to be 1.05 and 0.95 for cations and anions, respectively. The value for the volume of the posterior chamber (V_h) has been estimated from studies of histologic sections;[27] that for the area of the posterior chamber–vitreous interface has been estimated to be 1.1 cm.^{2}; the value for the diffusion rate within the vitreous (D) has been shown to be the same as through a salt solution having approximately the same concentration as the vitreous.[22]

The 2 unknowns in the equation are the concentration in the secreted fluid (C_s) and the value for the coefficient of diffusion, ($k_{d.ph}$). While neither of these terms is known, it is possible, nevertheless, to solve the single equation because of the fixed relationship they bear to each other. Since the value for $\frac{dC_h}{dt}$ is equal to zero under steady state conditions, the relationship between C_s and $k_{d.ph}$ can be obtained from the known concentrations in the aqueous of the posterior chamber and in the plasma, and from the flow rate, if the assumption is made that the concentration in the vitreous is equal to that in the posterior chamber, or if the concentration in the vitreous lying next to the posterior chamber is known. The lines in figure 7 show the relation between C_s and $k_{d.ph}$ for Na and Cl, assuming (as is only approximately true, see table 1) that the concentrations in the posterior chamber and vitreous at steady state are equal. It is apparent that the greater the value for $k_{d.ph}$, the greater the difference between the concentration in the posterior chamber and that in the secreted fluid.

The major factors which control the accumulation of a substance in the anterior chamber are:

1. The concentration of a substance in the fluid flowing from the posterior chamber.
2. The rate of flow of the fluid in and out of the anterior chamber.
3. The rate of diffusion between blood and anterior chamber.

Table 3
Numerical Values Used in Solving Text Equations

A_{hv}=1.1 cm.^{2}	k_{fh}=.06 min.$^{-1}$
D_{Na}=0.92×10^{-5} cm.^{2}/min.	V_a=.25 cm.^{3}
D_{Cl}=1.38×10^{-5} cm.^{2}/min.	V_h=.055 cm.^{3}
k_{fa}=.013 min.$^{-1}$	V_v=1.5 cm.^{3}

4. The diffusional exchange between anterior chamber and cornea.

The proportion of a substance entering by diffusion from the iris and by flow from the posterior chamber can be approximated from an analysis of the time course of the entrance of the substance into the aqueous from the plasma.

Friedenwald and Becker,[21] on the basis of data reported by Maurice,[4] have estimated that the diffusional exchange between the aqueous of the anterior chamber and the cornea could account for only about 10 to 15 per cent of the total turnover of Na^{24} in the anterior chamber. The magnitude of the exchange between anterior chamber and lens has not been estimated but is probably less than that between this chamber and the cornea. In the absence of similar information concerning Cl^{36}, no account will be taken in the computations which follow of the exchange between the cornea and the anterior chamber for either ion. If the appropriate correction were to be applied, it would have the effect, at least for Na, of increasing the coefficients for flow and diffusion by approximately 10 to 15 per cent each.

If we neglect flux to the cornea, the accumulation of a substance in the anterior chamber is described by equation (3):

$$\frac{dC_a}{dt} = k_{fa}(C_h - C_a) + k_{d.pa}(C_p - \alpha C_a). \quad (3)$$

The equation says that the rate of change of concentration of any substance in the anterior chamber is equal to the flow rate times the difference in concentration between that existing in the posterior and anterior chambers, plus the diffusion rate times the difference in concentration of the substance present in the plasma water and the effective concentration in the anterior chamber.

All of the terms are known except the diffusion rate between plasma and the anterior chamber, and it is this which will be evaluated.

The rates of accumulation of Na^{24} and Cl^{3} in the posterior and anterior chambers of the eye of the rabbit following the parenteral administration of these substances are shown in figures 8 and 9; the concentrations of Na^{24} and Cl^{36} in the plasma are also shown. The lines through the points were plotted by means of the analog computer, using the numerical values for the terms of equations (2) and (3) shown in table 3.

The values for the diffusion and flow coefficients for the posterior and anterior chambers, and the concentrations in the secreted fluid which correspond to the data, are given in table 4. The relative units of concentration, as shown in figures 8 and 9 and the fifth column of the table, were converted to ordinary units of concentration (mM/Kg. H_2O) on the basis of analytic data obtained previously by the author.[9] The concentration of Na in the secreted fluid which corresponded most closely was found to be slightly in excess of that in the aqueous of the posterior chamber; the concentration of Cl was significantly below the concentration in the aqueous. The data show that two-thirds of the Na^{24} enters the posterior chamber by secretion and one-third by diffusion, whereas less than one-third of the Cl^{36} enters by secretion, and over two-thirds by diffusion. The turnover rate of Na^{24} in the posterior chamber is 9 per cent and that of Cl^{36}, 21 per cent of the volume of this chamber per minute.

Approximately equal quantities of Na enter the anterior chamber by flow from the posterior chamber and by diffusion through the iris vessels. Significantly more Cl enters by diffusion than by flow. The difference in the magnitude of the diffusion rates for Na and Cl is approximately proportional to their diffusion rates in water. The turnover rates in the anterior chamber are 2.6 per cent and 3.1 per cent per minute for Na and Cl, respectively.

The rate of accumulation of total CO_2[28] following its parenteral administration is shown in figure 10. The concentration of $HC^{14}O_3$ in

Table 4

The Coefficients of Transfer by Diffusion and Flow in the Posterior and Anterior Chambers and the Concentration of Sodium and Chloride in the Secreted Fluid Entering the Posterior Chamber Which Were Used to Produce a Fit to the Experimental Data*

	Posterior chamber		Anterior chamber		Concentration secreted fluid (C_s)	
	Flow ($k_{f.h}$)	Diffusion ($k_{d.ph}$)	Flow ($k_{f.a}$)	Diffusion ($k_{d.pa}$)	Relative units	mM/Kg. H_2O
Na^{24}	.06	.03	.013	.013	98	146.5
Cl^{36}	.06	.15	.013	.018	47	53

*The units are reciprocal time (min.$^{-1}$).

ıe posterior chamber approached the steady ıate level (which incidentally, in the rabbit, ıut not in man, lies above that in the plasma) ɔ rapidly that the rate of penetration cannot ıeadily be determined.

Dr. Reddy and I believe that the explanaıion for the apparent rapid turnover rate of ıicarbonate lies in the fact that the turnover ıf total CO_2 between plasma and aqueous ıumor, as determined by isotopically labeled ıicarbonate, involves not only the entrance of total CO_2 contained in the secreted fluid, ıut the separate rates of diffusional exchange ıcross the blood-aqueous barriers of free CO_2, ıicarbonate ion, and undissociated carbonic ıcid. Unlike the situation for Na and Cl, the ıharacteristics of the blood-aqueous barriers ıre not the sole factors controlling the turnover rate. For the bicarbonate system, the rate of conversion of CO_2 to HCO_3 also influences the turnover rate.

Normally, free CO_2 diffuses rapidly from the ılood across the stroma and epithelium of the ıiliary processes or, in the instance of the ınterior chamber, through the iris vessels. In ıransit, labeled CO_2 exchanges for unlabeled CO_2 where the former is hydrated to form ıabeled HCO_3. The bicarbonate ion, in effect, ıecomes "trapped" in the aqueous humor to ıhe extent that its diffusibility into the blood ıs less than that of free CO_2. Catalyzed by ·arbonic anhydrase, the conversion of labeled CO_2 to labeled HCO_3 probably occurs at a ·ery rapid rate in normal animals. This reıction has the effect of reducing the concenıration of labeled CO_2 available to diffuse back ɔ the blood. The trapping action tends to ıaintain the gradient of free $C^{14}O_2$ between blood and aqueous close to that prevailing initially until the total labeled CO_2 has almost reached steady state level.

Green and Sawyer,[29] who have performed similar studies on the accumulation of HCO_3 in the aqueous, take a different view and believe that it is the bicarbonate ion itself which penetrates rapidly to the posterior chamber and that the conversion of CO_2 to HCO_3 does not contribute appreciably to the bicarbonate concentration in the aqueous humors. The present author finds it difficult to understand why the bicarbonate ion, which is larger than Cl and has the same charge and solubility characteristics, could enter the posterior chamber at least 10 times as rapidly as Cl. It should be noted, too, that administration of acetazoleamide 15 minutes prior to giving labeled HCO_3[28] decreases the rate of accumulation of total CO_2 by at least 10-fold—an effect readily explicable by the known inhibiting properties of this compound on the hydration of CO_2. In other words, the acetazoleamide suppresses the turnover through interference with the trapping mechanism.

The rate of accumulation of total labeled CO_2 in the aqueous humor of the anterior chamber is much lower than in the posterior chamber, but it is relatively more rapid than that of Na and Cl. Because the accumulation of total CO_2 in the anterior chamber, like that in the posterior chamber, depends on the separate rates of entrance of CO_2 and HCO_3 and also on their rates of interconversion, the rate of penetration of the bicarbonate ion itself remains unknown.

While the turnover rate of $HC^{14}O_3$ in the posterior chamber was too rapid to permit

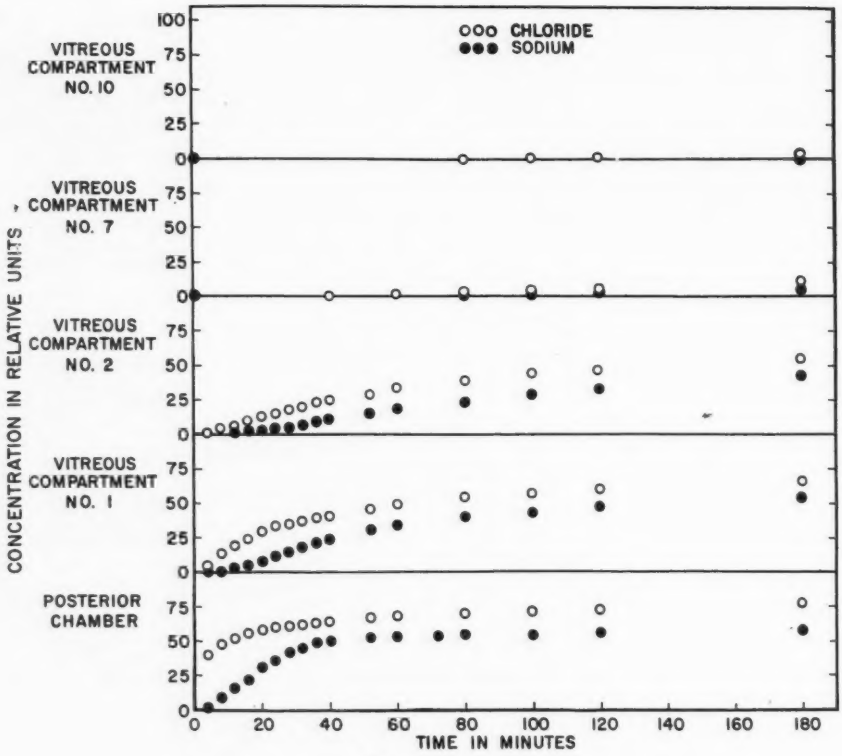

Figure 13

The concentration of Na^{24} and Cl^{36} in the posterior chamber and in several compartments of the vitreous model as determined with an analog computer. (Republished by permission of Documenta Ophthalmologica.[19])

evaluation of the concentration of this ion in the secreted fluid by the same method of treatment given Na and Cl, its concentration was estimated on the basis of the following assumptions and rationale.

The total concentration of cations is approximately equal to that of Na and K. It is assumed that the concentration of K in the secreted fluid is approximately equal to that in the plasma. About 90 per cent of the anion concentration consists of Cl and HCO_3; the remainder consists mainly of lactate and ascorbate. Because lactate is a relatively large ion, it appears probable that this ion will be present in the secreted fluid in a still more deficient state than Cl, i.e., about one-half of the concentration in the plasma, or 4 mM per Kg. of water. Ascorbic acid is thought to enter the posterior chamber chiefly by secretion, hence the assumption that its concentration in the secreted fluid is about 1.5 mM per Kg. of water, or just slightly higher than that in the aqueous of the posterior chamber. No allowance is made for PO_4, SO_4 or amino acids.

Table 5 shows the estimated ionic composition of the fluid secreted into the posterior chamber. For comparison, the composition of the aqueous humor of the posterior chamber and plasma as obtained analytically is also shown.

The concentration of the constituents of the secreted fluid differs from the concentration of the constituents of the plasma still more than does that of the posterior chamber, th

ost striking difference being the high concentration of HCO_3 and the relatively low concentration of Cl. The contrasting situation with regard to the concentration of HCO_3 and Cl in the secreted fluid and humors of the eye compared with plasma is shown graphically in figures 11 and 12. The numbers represent concentration in mM per Kg. of water.

While a detailed account of the several assumptions and sources of error involved in estimating the composition of secreted fluid is given elsewhere,[19] it is appropriate here to point out that the most serious source of error may be in the assumption that the volume flow of fluid in and out of the posterior chamber is the same. The values for the concentration of the ions in the secreted fluid, as shown in table 5, would be too low by an amount equal to the ratio of the volume of water entering to the volume of water leaving the posterior chamber by flow if this assumption were incorrect. Thus, if the bulk flow from the plasma into the posterior chamber were in reality but one-half that which flows out, the actual values for the concentration of the secreted ions would be twice those reported. It is noteworthy, however, that the relative concentrations for all the ions would be unaffected regardless of what proportion of the net amount of water enters by diffusion. Thus, the values reported for the concentration of the different ions in the secreted fluid must be considered minimal.

In calculating the concentration of Na and Cl in the secreted fluid, the assumption has also been made that the concentration of each ion is a constant linear function of its concentration in the plasma. This same assumption has been made tacitly in calculating the turnover rates of substances in the anterior chamber from data which show their rates of accumulation without taking into account the concentration of the substance present in the posterior chamber. The assumption introduces an error in these calculations because the concentration in the posterior chamber, like that in the anterior chamber, rises gradually and at times may actually reduce the concentration in the anterior chamber; e.g., see figure 8 for time periods after 70 minutes. Because of the minute distances involved in the ciliary processes, however, it seems likely that the composition of the secreted fluid at any moment may in fact bear a reasonably close linear relationship to the composition of the plasma. That is, there is probably an extremely rapid flux between the plasma and some intra- or extracellular compartment in the ciliary epithelium.

Table 5

Calculated Values for the Composition of the Fluid Secreted into the Posterior Chamber in Comparison with the Composition of the Aqueous Humor of the Posterior Chamber and of Plasma at Steady State

	Secreted fluid	Aqueous posterior chamber	Plasma
	mM/Kg. H_2O	mM/Kg. H_2O	mM/Kg. H_2O
Na^+	146.5	145	149.5
K^+	5	5	5
Total cations	151.5	—	—
Cl^-	53	100	112
HCO_3^-	93	34	24
$Lactate^-$	4	11	8
$Ascorbate^-$	1.5	1.3	.05
Total anions	151.5	—	—
pH	*7.95 est.	7.55	7.35

*Calculated on the assumption that the CO_2 tension of the secreted fluid is the same as that of the plasma. No allowance was made for organic acids present.

I believe that the data discussed in this paper help clarify the movement of ions into and out of the posterior chamber. At the same time, however, these data pose fundamental questions concerning the intimate mechanism of the formation of a secreted fluid in the ciliary epithelium; in the rabbit this fluid consists largely of hypertonic sodium bicarbonate. It still appears necessary to postulate the existence of an active transport system for sodium, the source of energy for which remains unknown. Because of the greatly reduced distances involved in forming a fluid within a crypt or minute vesicle compared with the distance from the capillary to the posterior chamber, it appears that the diffusion rate of water may no longer limit its osmotic transfer. If so, the need for postulat-

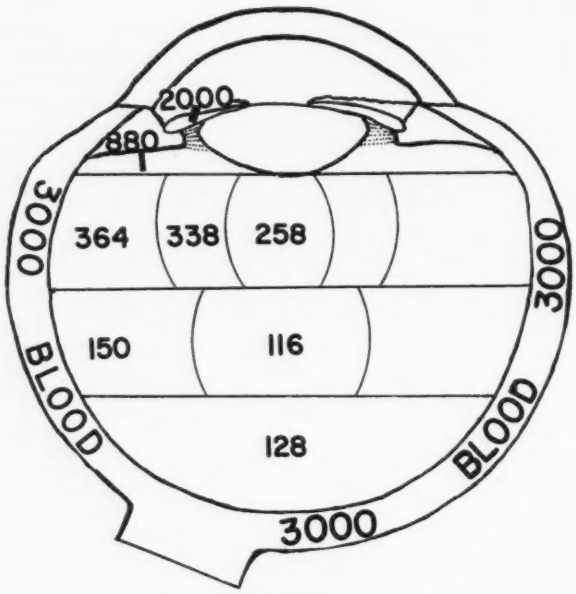

Figure 14

Comparative concentration of Cl^{36} in the plasma, posterior chamber and various parts of the vitreous humor determined experimentally. The numbers represent counts per minute.

ing an active transport mechanism for water is obviated.

Vitreous Humor

As stated previously, the solution of the system of simultaneous differential equations describing the loss of Na and Cl to the vitreous not only permits the determination of the total loss of the labeled ions from the posterior chamber, but also provides quantitative information concerning the rate of filling of each of the several compartments of the vitreous. The lower pair of curves of figure 13 represents the filling of the posterior chamber; the lines lying above these curves show the filling of the first, second, seventh and tenth compartments, in that order. The differences in the rates of filling the various portions of the vitreous illustrate the effect of lack of mixing.

Figure 14 shows the number of counts per minute in the blood, in the posterior chamber, and in various parts of the vitreous 35 minutes after the parenteral administration of Cl^{36}. The data indicate that Cl^{36} enters the vitreous more rapidly through the posterior chamber than through the retina. Moreover, the experimental data are in excellent agreement with the calculated values, e.g., there are 880 counts per minute per unit volume in the most anterior portion of the vitreous, or approximately 30 per cent of that in the plasma, while the calculated value for compartment 1 (fig. 13) is 35 per cent of that in the plasma.

Figure 15 is a log plot of the per cent of the total Na^{24} (broken line) and Cl^{36} (solid line) expected to be lost from the posterior chamber to the whole of the vitreous. Both curves have been adjusted to start at 100. Differences between 100 and the values shown indicate the percentage, at a given time, of the total amount which is expected to enter the vitreous by the time steady state conditions prevail. The rate of loss varies, being greater during the first several hours. If the vitreous were well mixed, the rate of loss would be constant throughout the whole time course.

In the mathematical model of the vitreous, no provision was made to take into account

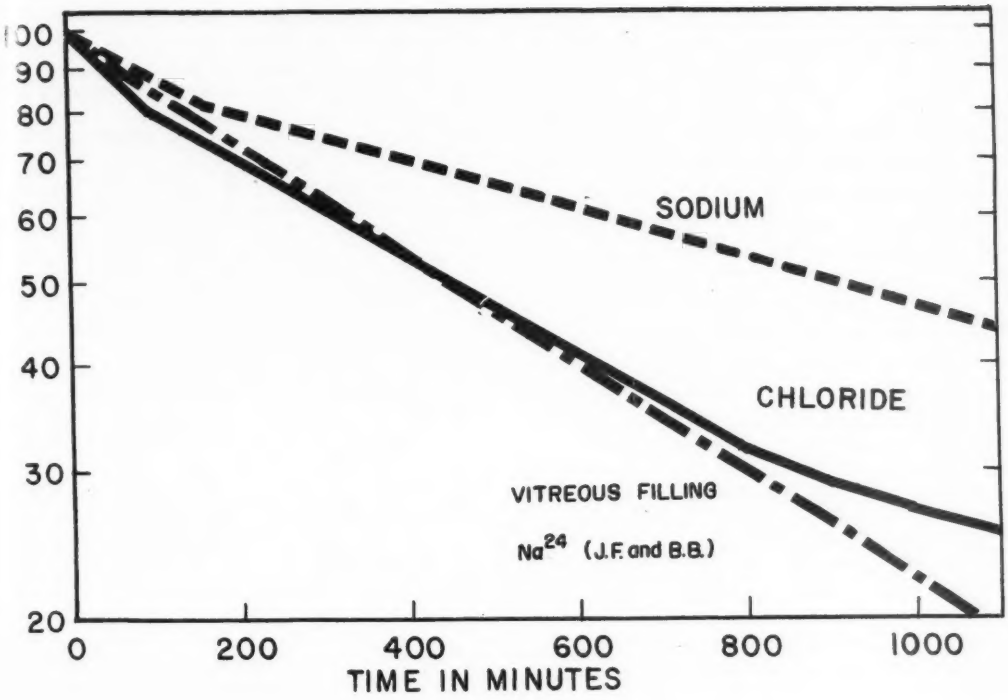

Figure 15

The percentage of the total amount of Na^{24} (broken line) *and Cl^{36}* (solid line) *expected to be lost from the posterior chamber to the vitreous at various times. The dot-dash line shows the filling of the vitreous following parenteral administration of Na^{24} as determined by Friedenwald and Becker.[21] (Republished by permission of Documenta Ophthalmologica.[19])*

the quantity of the labeled ions which may enter the vitreous from the blood through the retina. Maurice[22] has determined the total turnover of Na in the vitreous humor by injecting Na^{24} into the vitreous body of rabbits. He found that 60 per cent of the Na left by way of the posterior and anterior chambers and 40 per cent left by diffusion through the retina. Employing similar methods, Dr. Reddy and I have found that approximately one-half of the Cl^{36} exchanges with the blood through the retina. Friedenwald and Becker[21] have measured the concentration of Na^{24} in the vitreous following a single intraperitoneal injection. Their results are shown by the dot-dash line in figure 15. The difference between the dot-dash line, i.e., rate of loss to the vitreous, and the broken line presumably represents exchange through the retina, and corresponds to the results obtained by Maurice.[22]

Movement of ions into and out of the vitreous has been shown to occur at an appreciable rate; such movement not only greatly affects the rate of accumulation of these ions in the aqueous of the posterior chamber, but together with movement of other substances, undoubtedly serves a metabolic function as well.

Conclusion

Ions enter the posterior chamber of the eye unidirectionally by bulk flow of a "secreted fluid" from the ciliary epithelium and bidirectionally across the epithelium by diffusion. Approximately two-thirds of the Na enters unidirectionally compared with less than one-third of the Cl. Bicarbonate also penetrates the posterior chamber by both

mechanisms, but because of the extremely rapid, but unknown, rates of diffusional exchange of CO_2 and subsequent hydration the proportion which enters by each means cannot readily be determined.

There is a similar dual mode of entrance of ions into the anterior chamber; they enter by flow from the posterior chamber and by diffusion across the iris vessels. Half of the Na and approximately one-third of the Cl enter the anterior chamber by flow. No conclusions could be drawn concerning the relative amounts of bicarbonate entering the anterior chamber by flow and diffusion, but the rate of accumulation of total CO_2 is more rapid than that of Na or Cl.

The concentration of the various ions in the secreted fluid differs more from the concentration of these ions in the plasma than does that of the aqueous humor of either the posterior or anterior chambers. The fluid appears to consist, in the rabbit, chiefly of $NaHCO_3$ and NaCl; the concentration of HCO_3 was estimated to be almost 4 times that found in plasma.

The mode of formation of the secreted fluid is not known, but the probable hypertonicity of the fluid indicates that the mechanism involves a sodium pump and probably a diffusional transport of water as a result of osmotic forces.

There is a diffusional movement of ions between the posterior chamber and the vitreous humor which for Na and Cl is roughly equal in magnitude to the diffusional movement which occurs between the vitreous humor and plasma.

Acknowledgment

Acknowledgment is made to Dr. D. V. N. Reddy for his many helpful suggestions and criticisms.

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Starling's Law

At any given time, there must be a balance between the hydrostatic pressure of the blood in the capillaries and the osmotic attraction of the blood for the surrounding fluids. With increased capillary pressure there must be increased transudation, until equilibrium is established at a somewhat higher point, when there is a more dilute fluid in the tissue-spaces and therefore a higher absorbing force to balance the increased capillary pressure. With diminished capillary pressure there will be an osmotic absorption of salt solution from the extravascular fluid, until this becomes richer in proteids; and the difference between its (proteid) osmotic pressure and that of the intravascular plasma is equal to the diminished capillary pressure.

Here then we have the balance of forces necessary to explain the accurate and speedy regulation of the quantity of circulating fluid.—E. H. Starling. *Absorption by Blood Vessels.* J. Physiol. **19**: 312, 1895.

Electrolyte Excretion in Bile

By HENRY O. WHEELER, M.D., OSWALDO L. RAMOS, M.D., AND ROBERT T. WHITLOCK, M.D.

The high concentration of certain test substances in both urine and bile suggests that similarities exist between the biliary and renal tubular functions. Evidence from dogs with permanent duodenal fistulas indicates that the rate of elaboration of bile depends primarily on the rate of secretion of bile salts. The variations in flow and composition of hepatic bile seem to result from the addition of a fluid which is similar in some respects to pancreatic juice.

IN COMMON with other extrarenal secretory structures, the biliary tract apparently depends upon energy-consuming cellular processes rather than upon a hydrostatic filtration system for the production of its characteristic effluent. Nevertheless, there appear to be certain rather fundamental similarities between biliary and renal tubular function. These similarities become apparent when one considers the number of compounds which are secreted in high concentrations in both urine and bile. Examples of such compounds are phenolsulfonphthalein,[1] fluorescein,[2] p-aminohippurate,[3] penicillin,[3] and phlorhizin.[4]

It is noteworthy that among the substances which are most actively secreted into the bile are included the most potent known choleretics. The correlation between active secretion and choleretic potency may well have important implications with regard to the general nature of bile formation. Outstanding choleretic substances are the natural bile salts and their synthetic derivatives, cinchophen[5] and, to a lesser extent, the phthalein dyes such as Bromsulphalein, phenol red and bromcresol green.[1] All of these, it should be noted, are organic acids. Of these choleretic compounds, the natural bile salts are, of course, normally present in abundance and are therefore of the greatest physiologic interest.

The 2 major bile acids in the dog are the di- and tri-hydroxy cholanic acids, deoxycholic and cholic acid; of the 2, the latter is the more abundant. These bile acids represent the major end-product of cholesterol metabolism.[6, 7] In the dog and in other carnivores they are conjugated with taurine. Because of the free sulfonic acid group of taurine, the resulting conjugates are completely dissociated and highly soluble in the physiologic pH range, having a pK of about 1.5. These 2 bile salts, which can be measured accurately by the method of Mosbach et al.,[8] will be referred to hereafter simply as "taurocholate."

In the intact animal, the bile salts undergo extensive enterohepatic recirculation so that, as shown in figure 1, approximately 85 to 90 per cent of the bile salt in the bile at any given time represents material previously excreted and reabsorbed from the bowel.[9] The remainder represents new bile salt synthesized by the liver. The phenomenon of recirculation was described as early as 1870 by Schiff, and in the course of his experiments he also observed that bile itself, when introduced into the duodenum, was the most potent available stimulant for increased bile production.[10] It will be obvious, then, that interruption of this enterohepatic cycle must have major physiologic consequences which have to be considered in the planning and interpretation of any study in which bile is removed from the experimental subject and not replaced.

Time does not permit a review of the many ingenious technics which have been developed for repeated collections of bile from unanesthetized animals. The particular device we have employed is a duodenal cannula developed by Thomas.[11] This apparatus, which is held in the duodenum by a hard rubber flange, permits the creation of a permanent duodenal fistula opening directly over the ampulla of

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, N. Y.

V ter. Between studies it is kept stoppered, b t during an experiment it is opened, and a s all ureteral catheter is inserted directly t ough the ampulla of Vater and advanced w ll into the common duct. Except for the f t that the animals are cholecystectomized, t is technic leaves the biliary tract in normal c ndition between studies and free of per- n nent foreign bodies. All of the studies v ich will be considered in this paper were c nducted on 4 such dogs. In each study, the d g was held in the upright position by means o a sling. Bile was collected either by gravity o with the assistance of gentle suction pro- v ded by a tuberculin syringe.

When bile is collected continuously over a period of hours, there is progressive diminution in bile flow because of the interruption of enterohepatic circulation of bile salts. Figure 2 illustrates this phenomenon and also certain general features of bile composition with which we shall be concerned. At the top is shown bile flow in ml./min., in the middle is the pH, relative to a line drawn at 7.4, at the bottom is shown the electrolyte composition of individual bile specimens. In each block diagram the major cations, sodium and potassium, are on the left and the anions, taurocholate, bicarbonate and chloride, are on the right. The specimen of bile labeled C_D is typical of bile removed from the common duct at the moment of catheterization. We shall shortly examine this type of bile in more detail. It should be noted here, however, that, in contrast to subsequent samples of flowing hepatic bile, the concentration of taurocholate in "common duct bile" is very high, the total ionic concentration is high, the pH is low, and there is very little bicarbonate or chloride. Over the course of 2 to 3 hours there is progressive diminution in bile flow and also in taurocholate concentration. Thus, in this type of experiment the output of all solutes, but particularly the output of bile salt, diminishes as expected. Not only is the electrolyte composition of bile difficult to interpret under these circumstances, but actually it is often difficult even to obtain bile in sufficient quantity for analysis. It was found that this "unsteady" state can be averted by the constant

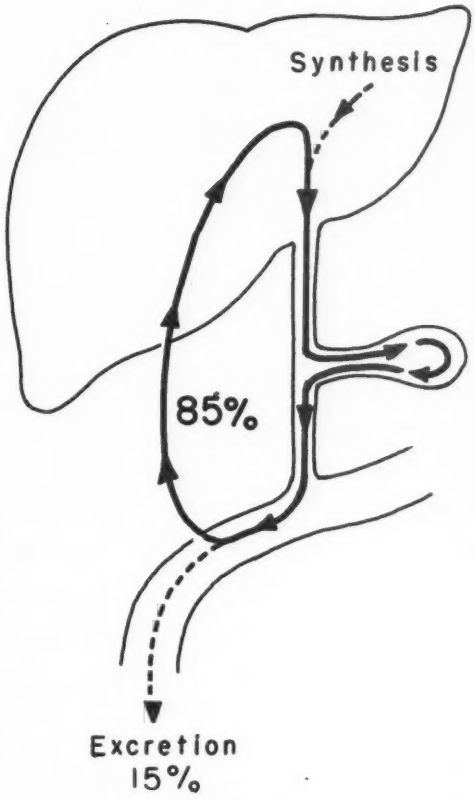

Figure 1

Enterohepatic circulation of bile salts. About 85 to 90 per cent of the bile salt excreted into the duodenum is reabsorbed and returned to the liver by way of the portal vein.

intravenous replacement of bile salt. Before proceeding to the results which were obtained by using this technic, let us examine more closely the composition of "common duct bile."

The specimen on the left of figure 3 is typical of many specimens of "common duct bile" obtained upon catheterization of fasting dogs. Presumably it represents bile which was formed over a period of several hours prior to catheterization and held in the duct system by the normal action of the sphincter of Oddi. In the center is shown the composition of canine gallbladder bile, and on the right, for contrast, that of canine plasma. There is obviously a striking similarity between "com-

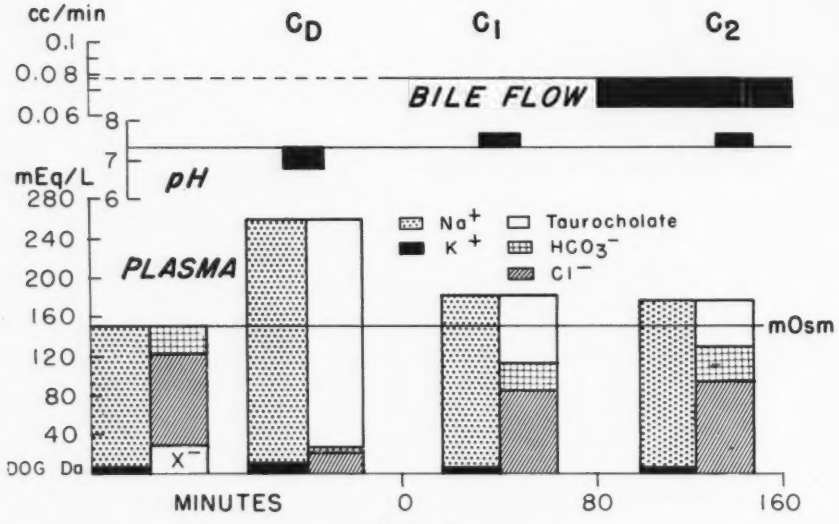

Figure 2

Bile flow and composition during continuous collection. Bile withdrawn from the common duct immediately after catheterization (C_D) has a high concentration of taurocholate and low concentrations of chloride and bicarbonate. During continuous collection, without bile salt replacement, the flow of bile and the concentration of taurocholate diminish progressively. Composition of plasma is shown at left for comparison.

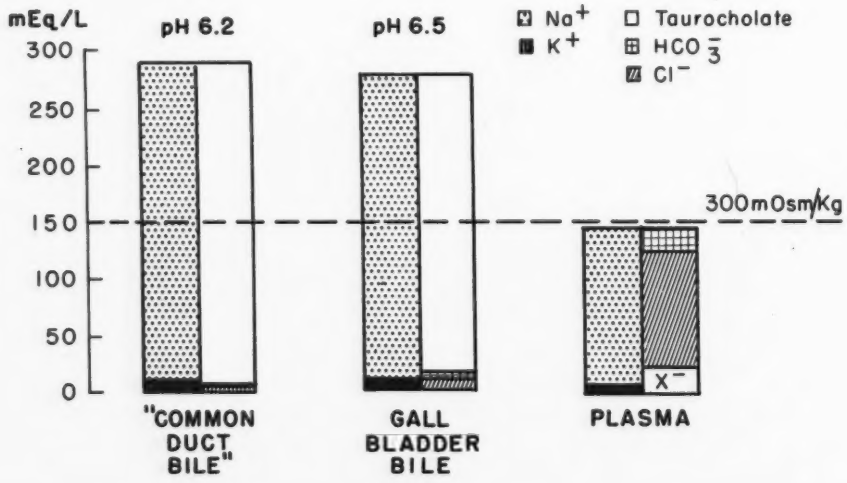

Figure 3

Comparison of typical fasting canine "common duct" and gallbladder bile. Bile retained in the bile ducts by the sphincter of Oddi is similar to gallbladder bile in that it has a high concentration of taurocholate, low concentrations of chloride and bicarbonate and a low pH. Note that the total ionic concentration is high, although osmolality is equal to that of plasma (at right). (Republished by permission of the Journal of Clinical Investigation.[16])

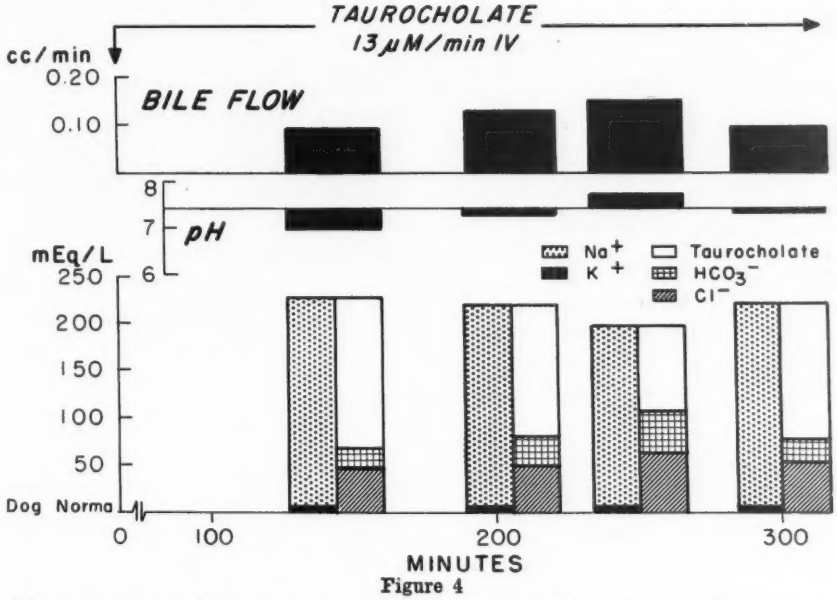

Figure 4

Bile composition and flow during constant intravenous infusion of taurocholate. The rate of bile salt excretion is constant, but there are spontaneous variations in flow and electrolyte composition. Highest flows are accompanied principally by increased pH and concentration of bicarbonate.

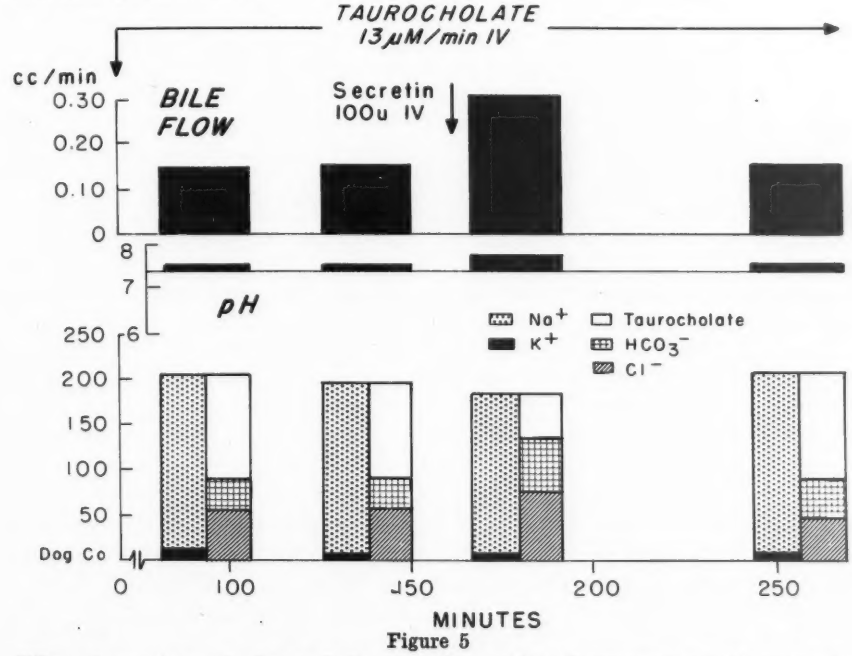

Figure 5

Effect of secretin on the flow and the composition of bile. Intravenous administration of secretin causes a marked choleresis and a very high pH and bicarbonate concentration. The excretion of bile salt is unaffected. (Republished by permission of the Journal of Clinical Investigation.[16])

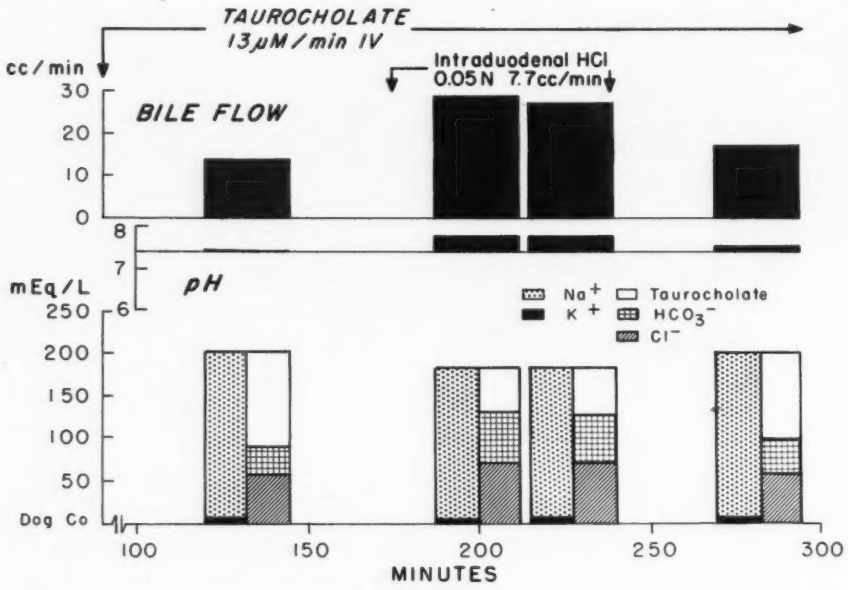

Figure 6

Effect of intraduodenal infusion of hydrochloric acid. The choleresis and changes in electrolyte composition are similar to those produced by exogenous secretin and are probably attributable to release of endogenous secretin.

mon duct bile'' and gallbladder bile in that, so far as anions are concerned, each bile is practically a pure solution of bile salt. It seems quite probable that, at least in cholecystectomized dogs, the common duct and its major branches serve in effect as a gallbladder in concentrating the bile salts by removal of other solutes and water.

The osmolality of bile, according to all reports and under all the circumstances we shall discuss, is very close to that of plasma (that is, about 300 mOsm./Kg.). In all bile specimens, however, and particularly in those of the type illustrated in figure 3, the total ionic concentration (sum of anions plus cations) is much greater than 300 mEq./L. This marked discrepancy between ionic concentration and osmolality can be attributed to the fact that taurocholate, like many other surface-active substances, forms large multipolar aggregates, or micelles, when its concentration exceeds a critical value known as the ''micelle point.'' The ''micelle point'' of pure taurocholate, as determined by Pethica and Schulman,[12] is about 0.007 M, which is much lower than the concentration of taurocholate in bile. Thus, the taurocholate ion itself is virtually inactive osmotically. Its osmotic significance is, in effect, attributable solely to the cation which must accompany it to preserve electroneutrality. Consistent with this view is the finding that, regardless of taurocholate concentration, virtually all the osmotic activity of any bile specimen may be accounted for as the sum of sodium, potassium, chloride and bicarbonate.

To return to the composition of flowing hepatic bile, figure 4 illustrates a typical study in which sodium taurocholate was infused intravenously at a constant rate of 14.5 μM/min. In this and similar studies, such a constant infusion resulted in stabilization of taurocholate excretion at a constant rate approximately equal to the rate of infusion. There was no *progressive* diminution in bile flow. Nevertheless, as shown in figure 4, sig-

n ficant fluctuations in bile flow often oc- c rred. There was a reciprocal relationship b tween bile flow and taurocholate concentra- t n, consistent with the constant output of t is constituent. There were also characteris- t c changes in other electrolytes. As flow in- c eased, the chloride concentration increased s ightly, but there was a more striking in- c ease in bicarbonate concentration and a cor- r sponding rise in pH.

When 100 units of the intestinal hormone, s cretin, was administered intravenously (fig. 5), a marked increase in bile flow occurred, e ceeding the highest spontaneous flows ob- served in the same animal. The excretion rate of taurocholate was unchanged, but the secre- tin choleresis was accompanied by a very high concentration of bicarbonate (reaching values as great as 60 mEq./L.) and pH (to as high as 7.8).

The intraduodenal infusion of hydrochloric acid is known to stimulate the release of en- dogenous secretin.[13] As shown in figure 6, this maneuver resulted in a change similar to that produced by exogenous secretin. There was an impressive choleresis accompanied, once again, by a high bicarbonate concentration and pH. As with exogenous secretin, these changes occurred in the face of a constant rate of taurocholate secretion.

In each animal there was a reproducible relationship between bile composition and bile flow as shown in figure 7. Whenever bile flow increased, whether spontaneously or under the influence of secretin (on the extreme right), there was a marked increase in pH and concentration of bicarbonate. The concentra- tion of chloride also increased, but this change was small compared to the increment in bicar- bonate. In contrast to these changes, the en- tirely different effect of intravenous acetazole- amide is also shown in figure 7. This agent, in a dose of 60 mg./Kg., resulted in a choleresis which was characterized by a relatively high chloride concentration and a comparatively low bicarbonate concentration and pH.

All of the studies to which we have alluded were conducted with the rate of secretion of taurocholate arbitrarily fixed at about 15

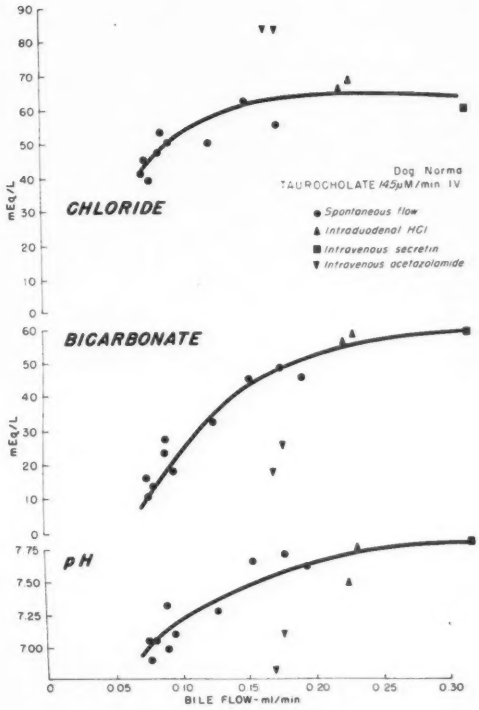

Figure 7

Relationship between bile flow and composition during constant infusion of taurocholate. At higher rates of flow, the concentrations of bicarbonate and chloride and the pH were increased. The increment in bicarbonate was greater than that in chloride. Highest flows were observed after intravenous administration of secretin or intra- duodenal administration of hydrochloric acid. Acetazoleamide produced a choleresis in which chloride was the predominating anion. (Repub- lished by permission of the Journal of Clinical Investigation.[16])

μM/min. It should be mentioned, however, that we have observed a very similar qualita- tive relationship between bile composition and flow at a taurocholate secretion rate of about 40 μM/min.—although, of course, the absolute values of bile flow were much higher than those shown here. It is also worth noting that the arbitrary rate of taurocholate secre- tion employed in the present studies repres- ents only about one-tenth of the maximal rate at which the liver is apparently capable of secreting this bile salt. The transport maxi-

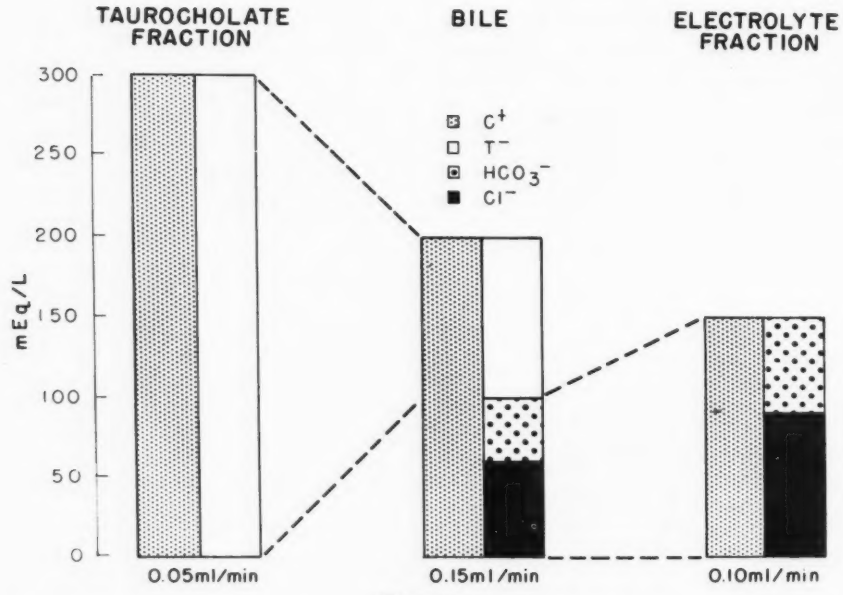

Figure 8

Hypothetic fractions of bile. The concentrations and flows are based on the assumption that each solution is isosmotic with respect to plasma. When the output of taurocholate is constant, all variations in bile flow and composition can be attributed to changes in flow and composition of the "electrolyte fraction." (Republished by permission of the Journal of Clinical Investigation.[16])

mum for taurocholate is well over 100 μM/min. in dogs of this size.

One way of explaining the observed variations in bile flow and composition would be to postulate that bile is formed by the admixture of a number of solutions which differ from one another in composition and mode of production. With this thought in mind, we have arbitrarily elected to regard each bile specimen as a mixture of 2 hypothetic isosmotic solutions as shown in figure 8. On the left is a pure solution of taurocholate which—because of the associative properties of the taurocholate ion—would be isosmotic at a concentration of about 300 mM/L. On the right is a solution of chloride and bicarbonate which we shall call the "electrolyte fraction"; the sum of these ions would be equal to about 150 mEq./L. for an isosmotic solution. Thus, on the basis of the osmotic behavior of all these constituents, it is possible to calculate, for each bile specimen, the flow and composition of these 2 hypothetic constituents. Under the conditions we have employed, the output of the "taurocholate fraction" is maintained at a constant rate. Hence, the changes in bile flow and composition must be attributed to changes in the output and composition of the "electrolyte fraction," and we shall therefore examine these changes.

When one examines the relationship between composition and output in the "electrolyte fraction" (fig. 9), it is apparent that increasing output is accompanied by reciprocal changes in chloride and bicarbonate concentration. At the very highest rates of flow —after secretin administration—the concentration of bicarbonate achieves its highest value of about 75 mEq./L. and chloride concentration reaches a minimum at about the same level. This figure bears a striking resemblance to illustrations of the behavior of pancreatic secretion[14] although, of course, much higher concentrations of bicarbonate and lower con-

centrations of chloride have been observed in pancreatic juice under maximal secretin stimulation. Note again the fact that acetazoleamide administration results in high concentrations of chloride and low concentrations of bicarbonate.

The results of these studies are consistent with the view that at least 2 processes are involved in the elaboration and modification of bile. First, it is obvious that the rate of bile production is profoundly affected by the rate of secretion of a number of substances of which the bile salts are of the greatest physiologic importance. In spite of the variations noted there is, in fact, a rough proportionality between total bile flow and rate of bile salt secretion. Also, as noted earlier, bile flow becomes almost vanishingly small in the animal which is acutely deprived of recirculating bile salt unless the bile salt is replaced by another route. It would seem entirely reasonable to postulate that the primary event in bile formation is the active secretion of bile salts and, to a lesser extent, of certain other substances. The addition of water and many diffusible constituents could then occur passively along the resulting osmotic and electrochemical gradients. This viewpoint has been enunciated in a recent review by Sperber[15] with which our data are wholly in accord.

The second process involves the modification of the final composition of bile by the addition—at an unknown site in the biliary tract—of a solution which is similar in many respects to pancreatic juice. This bicarbonate-rich fluid appears to be responsible for the spontaneous variations in bile flow which occur in spite of the constant rate of secretion of bile salts, and its output is maximal following stimulation by exogenous or endogenous secretin.

Finally, the possibility of reabsorptive mechanisms in the bile ducts must not be overlooked, although at present the only evidence of the existence of such mechanisms is that which can be inferred from the similar composition of gallbladder bile and bile resting in the common duct.

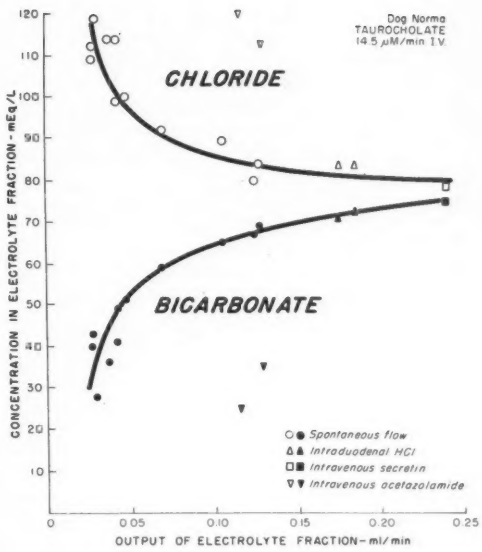

Figure 9

Composition of "electrolyte fraction" during changes in its output. A reciprocal change in bicarbonate and chloride, similar to that observed in pancreatic juice, is observed as output increases. After the administration of acetazoleamide, the concentration of chloride is high and the concentration of bicarbonate is low. (Republished by permission of the Journal of Clinical Investigation.[16])

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The Origin of Life

At first there were the simple solutions of organic substances, whose behavior was governed by the properties of their component atoms and the arrangement of those atoms in the molecular structure. But gradually as a result of growth and increased complexity of the new molecules new properties have come into being and a new colloid-chemical order was imposed upon the more simple organic chemical relations. These newer properties were determined by the spatial arrangement and mutual relationship of the molecules. Even this configuration of organic matter was still insufficient to give rise to primary living things. For this, the colloidal systems in the process of their evolution had to acquire properties of a still higher order, which would permit the attainment of the next and more advanced phase in the organization of matter. In this process biological orderliness already comes into prominence. Competitive speed of growth, struggle for existence and, finally, natural selection determined such a form of material organization which is characteristic of living things of the present time.—A. I. Oparin. *The Origin of Life*. Translated with annotations by S. Morgulis. Ed. 2. New York, Dover Publications, 1953, pp. 250-251.

Discussion

Dr. Welt: I should like to ask Dr. Farber if, in contrast to the behavior of sodium during the hyponatremia of sodium depletion, sodium leaves the cartilage during the hyponatremia produced by a water load.

Dr. Farber: That is a very interesting question to which I do not have the answer. We have not, as yet, caused dilutional hyponatremia; to date, our only experiment involves sodium depletion hyponatremia.

Dr. Robert S. Gordon: I would like to ask Dr. Farber a question: has he done experiments other than those recounted this morning, which would prove that it is specifically the sodium, and not the chloride, for example, which is held by chondroitin sulfate?

Dr. Farber: There is no evidence, as far as I know, to indicate that the activity of chloride ion changes in solutions of chondroitin sulfate. We haven't done any experiment, though, to test this hypothesis.

Dr. Ullrich: I have a question for Dr. Farber. As Preto-Parvis and Forni have shown, in the inner medullary region, there is a great deal of metachromatic substance. Have you any suggestions about its function?

Dr. Farber: I wish I had the answer to that. It is a very interesting situation. At the moment, Mr. Gluck in our laboratory is trying to isolate mucoprotein from various portions of the papilla of the rabbit, and very preliminary evidence indicates that, in the lower regions of the papilla, there is quite a bit of metachromatic material which behaves on the starch block like mucoprotein. Once this material is well-characterized we can get to the problem of whether it serves any function there. In other sections of the kidney there seems to be very little metachromatic material.

Dr. Mauro: Dr. Schmidt-Nielsen, have you ever measured the maximum hydrostatic pressure which is developed in the main tubules of the tear gland in the marine birds? I have in mind a comparison with pressures generated in ordinary salivary glands.

Dr. Schmidt-Nielsen: I'm sorry, but we have not made this measurement in marine birds.

Dr. Kinsey: I don't know whether or not this is relevant, but, in the eye, it is routine to measure pressures. There one finds pressures to be high, even of the order of 90 mm. Hg. One of the problems is, how does the eye get such high pressures, pressures which may be, for example, high enough to shut off the circulation?

Dr. Mauro: May I turn to another topic? Dr. Wheeler, what values do you obtain for the tonicity of the bile fluid measured *in situ*?

Dr. Wheeler: As we measure it, by freezing-point depression, it is approximately 300 milliosmols per kilogram. It varies over a rather narrow range; it is always practically indistinguishable from plasma.

Dr. Mauro: I asked that question because of Dr. Opie's work; he has found that in general the cells of the liver *in situ* have about twice the tonicity of plasma. So, if you take a piece of liver and put it into ordinary plasma or isotonic Ringer's, it usually swells.

Dr. Wheeler: Perhaps Dr. Leaf could answer this a little better than I. I believe that using his melting-point technic, we found that the liver was isotonic with respect to the plasma.

Chairman Taggart: Dr. Leaf, do you wish to comment?

Dr. Leaf: Yes, we think that the liver cells, like every other tissue which we studied in the mammal, except the renal medulla, are isotonic to the plasma. The discrepancy between our results and those of Dr. Opie may be due to the complicated nature of his experiments.

Dr. Bartter: Dr. Schmidt-Nielsen, how low have you found the tonicity of the bird's tears

to be? Can they actually weep hypotonic tears?

Dr. Schmidt-Nielsen: I suppose that you really mean the secretion from what I call the "salt gland." Birds also have tears which are isotonic. The salt gland secretion is rather species-specific, and is always hypertonic. The exact tonicity varies somewhat from species to species and may be influenced by experimental circumstances, such as the administration of cholinergic substances.

Dr. Gilman: I would like to invite Dr. Leaf to comment on the fact that energy for transport in the jejunum, the bladder, and the frog skin, comes from glycolysis. To me it is rather fantastic that the gut uses glycolysis, rather than oxidative metabolism, for transport.

Dr. Leaf: I was very interested to hear Dr. Gilman's evidence that glycolysis supplies the energy for ion transport in the small intestine. As a result of our studies on active sodium transport by frog skin and toad bladder and their energy metabolism, I have, perhaps, looked at this matter in a slightly different manner. It has been known for a long time, of course, that the red blood cell was capable of active ion transport, deriving its energy solely from glycolysis. On the other hand, the evidence seems equally good, I think, that muscles and nerve tissues depend largely upon aerobic metabolism for the energy of ion transport.

We were rather pleased when we found, in the toad bladder and frog skin, that either aerobic metabolism or anaerobic glycolysis could supply the energy for transport. Although the frog skin and toad bladder do transport sodium under completely anaerobic conditions, it is also true that the rate of sodium transport is somewhat less than under aerobic conditions. Usually the sodium current is one-fifth to one-half that under aerobic conditions; but, when we calculated the approximate number of sodium ions transported relative to the number of high-energy phosphate bonds synthesized, the "transport efficiency" was about equal under aerobic or anaerobic conditions. It seems, therefore, that these tissues can maintain active sodium transport effectively, whether the energy requirements for this process are derived from aerobic or anaerobic metabolism. Needless to emphasize, however, the efficiency with respect to the substrate, tissue glycogen, is much higher under aerobic conditions.

My friends chide me when I make any analogies between the toad bladder and the renal tubule, but I think it may be pertinent to note, in view of the very low oxygen tensions which Pappenheimer and co-workers have shown to exist in the renal medulla, that the renal tubule, or at least some portions of it, may have to operate under very low oxygen tensions. It is reassuring, therefore, to know that the process of transmembrane sodium reabsorption (which, I think, is the major transport process in the renal tubule) can also be maintained in the toad bladder efficiently by glycolytic sources of energy.

Dr. Gilman: That is very interesting, but our Chairman assures me that the kidney is very, very undistinguished as far as glycolysis is concerned. Secondly, if I am not mistaken, the kidney performs gluconeogenesis. Thirdly, the beautiful work of Chinard shows that glucose just goes right across the tubular cells and that no glycolysis is involved in its transport. On these grounds, one might be inclined to dismiss glycolysis as a source of energy for transport in the kidney.

Chairman Taggart: You may be putting me in an unfair position, Dr. Gilman. Insofar as certain proximal tubular functions which have been examined in vitro are concerned, added glucose does not appear to support transport, nor, if one measures rates of glucose disappearance, are there evidences of a very rapid rate of glycolysis. On the other hand, I could not argue against Dr. Leaf's contention that there may be segments of the nephron actively transporting sodium and that this transport may depend, to a considerable extent, on glycolysis. The studies to which I have referred concern cortical tissues only.

Dr. Ullrich: There are several types of observations which indicate that glycolysis is the source of energy for ion transport in the inner medullary region. First, there is a high rate of glycolysis in the inner medullary re-

gion, aerobic as well as anaerobic. Second, there is a high content of hexokinase and aldolase as well as a paucity of enzymes which are involved in oxidative processes. Finally, this same region contains a pool of glycogen and of phosphorylase activity but is without phosphatase activity.

Dr. Gilman: I don't know who is going to do the experiment first, but I dare say that we will all want to rush back and find out what inhibitors of glycolysis do to the osmotic ceiling of the urine.

Dr. Wakim: I should like to ask Dr. Wheeler if he encountered any hemolysis from the infusion of taurocholate and, if so, would that interfere with the ionic analysis?

Dr. Wheeler: Hemolysis was certainly one of the major difficulties that we expected to encounter. We have found that if we infused taurocholate in dextrose, in a concentration no greater than 1.5 per cent, we could infuse it at almost any rate without producing any physical hemolysis; so that is what we have done.

On Scientific Discovery

Keith Cannon has a story which, if it isn't true, should be. It seems that Barcroft was doodling with a pencil one day on a piece of log paper, happened to plot out some points of a CO_2 dissociation curve, to find that they fell precisely on a straight line. Entertained by this, he dashed off a letter to Henderson and told him about it. In due course a reply came. "Dear Barcroft," it read, "by intellectual processes of a low order, you have arrived at a conclusion of some importance."—D. W. Richards. *Lawrence Joseph Henderson.* Physiologist 1: 36 (Feb.), 1958.

V. EXTRARENAL REGULATION OF SALT AND WATER EXCHANGE

Chairman: George Sayers, Ph.D.

INTRODUCTION

By George Sayers, Ph.D.

Thus far we have been concerned with organs which transfer salt and/or water between sites within the organism or between the external environment and the organism. We now turn our attention to regulatory systems which modulate the rate or the direction of these transfers for the purpose of maintaining a relatively fixed composition and volume of the body fluids. The effective stimuli for the receptors of the regulatory systems may be classified into 3 general categories. Figure 1 is a grossly simplified illustration of the regulatory systems. Alterations in the composition of the body fluids stimulate chemoreceptors. Alterations in the volume or in the pressure of the body fluids stimulate volume or pressoreceptors. Finally, a third category of stimuli, pain, noise, with or without accompanying emotional reactions such as fear, anger, may activate a variety of visceral and endocrine organs. These stimuli are designated premonitory since in a sense they forewarn the organism of impending events.

Afferents transmit the messages from all 3 categories of receptors to the central nervous system wherein integration takes place to be followed by the elaboration of hormones which are carried in the systemic circulation to modulate the functional activity of the kidney, the gut, the sweat glands and other effector organs.

Figure 2 is meant to illustrate the neuro-

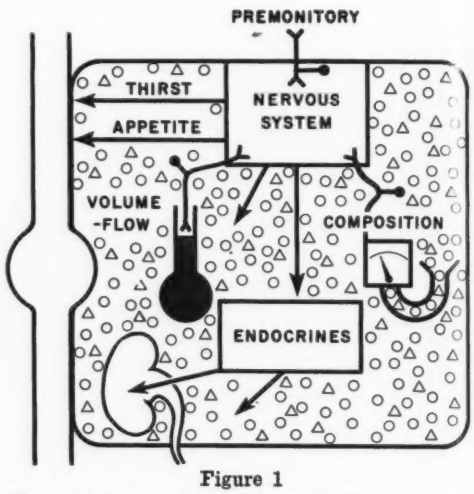

Figure 1

The regulatory systems which control the composition and volume of the body fluids.

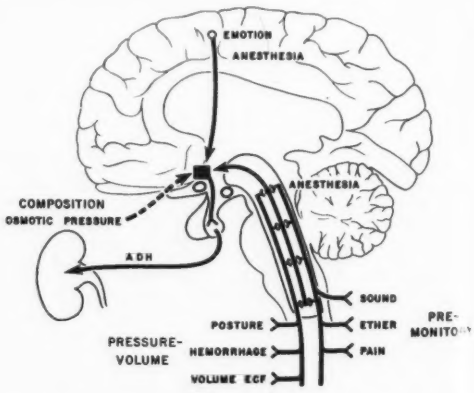

Figure 2

The neuroendocrine regulatory system for wate balance.

From the Department of Physiology, School of Medicine, Western Reserve University, Cleveland, Ohio.

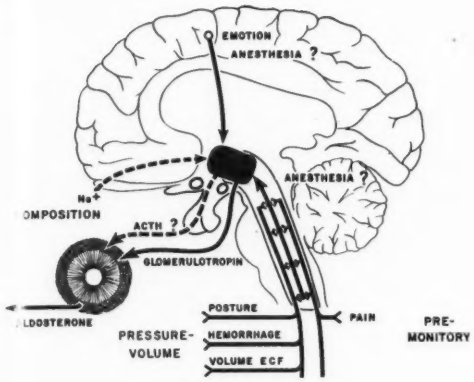

Figure 3

The regulatory system for the secretion of aldosterone.

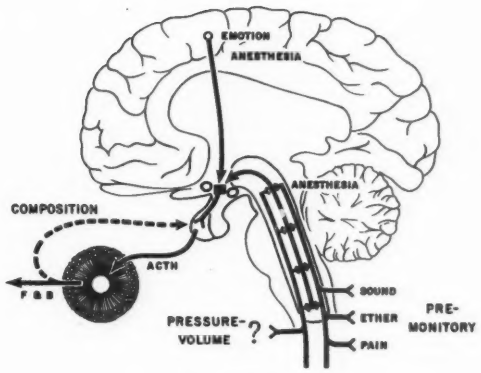

Figure 4

The regulatory system for the secretion of cortisol.

endocrine regulatory system which is concerned with water balance. Receptors, probably located in the path of the internal carotid artery, monitor osmolarity of the extracellular fluid and modify water excretion through the mediation of the antidiuretic hormone. Volume receptors, perhaps located in the atria of the heart, also modulate the rate of elaboration of ADH. Finally, premonitory stimuli acting upon a variety of sensory receptors may exert important influences upon water excretion by exciting release of ADH. Similar patterns of organization apply to aldosterone (fig. 3), and to cortisol (fig. 4). Of some interest are the striking similarities in general organizational pattern which exist, on the one hand, among these neuroendocrine-controlling mechanisms themselves and, on the other, to neurovisceral-controlling mechanisms, which modify, for example, blood pressure and respiration.

Perhaps this introduction will give some perspective for this afternoon's session during which we are to hear about recent advances in certain areas of the complex neuroendocrine systems which I have just outlined.

Volume Receptors

By Louis G. Welt, M.D.

This paper reviews the complicated interplay which may be involved in the retention of water and salt by the kidney. For the sake of brevity, it does not treat either the mechanisms which regulate the intake of water or the disposition of fluid throughout the body. The neurohumoral pathways by which a change in intravascular volume can effect a retention of water by the kidney receives particular attention. The difficulties in dissociating intrarenal from extrarenal determinants of salt and water excretion are considered in detail.

THIS REVIEW will deal with only 1 aspect of the problem of why, on the one hand, we don't shrivel to clay and dust, and, on the other, why we just don't blow up and bust. It will not attempt to discuss the problems which relate to the regulation of volume among the several major and minor fluid compartments. Nor will it consider either the important determinants which condition the distribution of volume within the vascular component itself, or the factors which determine our appetites for, and the ingestion of, salt and water. The problem at hand concerns only those factors that control the fluid volume, and how this volume is affected by the elimination of salt and water from the organism by the excretion of urine. This small, but still awe-inspiring, segment of the problem can be defined by the question: how are the kidneys apprised of and how do they respond to distortions of volume in such a fashion as to selectively augment or diminish the rate of excretion of salt and water, so as to maintain the volume of the body fluids within certain narrow limits?

The kidney's ultimate response must be either a primary change in hemodynamics or an altered rate of tubular reabsorption. These mechanisms could be local and automatic; they could be influenced by neural discharges or by humoral agents. If there is a receptor which is sensitive to some function of volume such as stretch, pressure, or flow, we must still look among these several alternatives for the efferent arc of a reflex. If there is a volume receptor, its presence certainly does not exclude other types of detectors. Lastly, if there is a volume receptor, there certainly need not be only one. It would indeed be surprising if this function of body fluid volume, which is so crucial to the very existence of the organism, were to be regulated by some simple single control mechanism. It is much more reasonable to approach this problem with the perspective that there are many devices through which an error in volume is appreciated, several means by which the kidneys are notified, and a variety of mechanisms by which the kidneys can compensate for the error.

The most basic of all mechanisms might very well reside within the kidneys themselves. It should not be forgotten that when the nerve supply of a kidney has been totally interrupted,[1] or when the supply of salt-retaining adrenocortical hormone can be neither increased nor decreased,[2] the kidney can, nevertheless, respond immediately and appropriately to a variety of procedures which appear to have, as the common denominator, a change in total volume or an alteration in its disposition.

The regulation of the excretion of water and the role of antidiuretic hormone have already been described in this symposium.[3] With respect to the antidiuretic hormone, it is apparent that the effective osmolality of the body fluids is the primary stimulus leading to the elaboration of a concentrated or a dilute urine.[4] However, osmolality is only 1 aspect of the composition of body fluids.

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A distortion of the volume of body fluids can also promote changes in the secretion of the antidiuretic hormone, but these appear to be of secondary importance.[5-9] Paradoxically, however, the best evidence for volume receptors is stretch receptors that appear to influence the secretion of antidiuretic hormone.[10-17] These will be referred to later.

The regulation of the excretion of salt will occupy our attention first, because the quantity of sodium in the body appears to be the primary influence with respect to the volume of fluid in the body. When salt is retained in excess of water, thirst is stimulated. Thirst, in turn, promotes the ingestion of water, and the secretion of antidiuretic hormone is stimulated, so that the urine which is elaborated is concentrated and small in volume. The quantity of salt that gains access to the urine is presumably the difference between that which is filtered at the glomerulus and that which is reabsorbed by the tubules, assuming, for the sake of simplicity, that there is no significant net secretion of sodium. The quantity of salt which is excreted, then, might readily be expected to be influenced by the filtered load, a value which is the product of the concentration of sodium in the filtrate and the volume of the filtrate.

There are, in fact, innumerable examples of a direct correlation between the change in the filtered load of sodium and the change in its rate of excretion.[18] Under most circumstances, the per cent of the filtered load which is excreted is small and, for this reason, one might readily account for many changes in the rates of excretion of salt on the basis of very small alterations in the filtered load.

Thus, at the very start, we are faced with the problem of how we can ever exclude with certainty a minor change in filtered load as the determinant of most changes in the rate of excretion of salt. This problem can sometimes be resolved, however, if the experimental design includes enough data so that a statistical treatment may, at least, either allow one to conclude that it was unlikely that a change in filtered load was responsible, or else force one to conclude that the alterations in excretion could have been due to concordant changes in filtered load.

Even if this problem were surmounted, there are still other matters concerning local renal hemodynamics that must be considered. There are approximately 2,000,000 nephrons in the 2 human kidneys, and it has recently been emphasized by Bradley and Wheeler[19] that all of these cannot be considered to be identical. One cannot assume that each glomerulus filters the same volume as each of the others. This has significance for several reasons.

The reabsorptive activity of a nephron may be, in part, conditioned by the volume of filtrate formed in that nephron. Furthermore, there are reasons to view the population of nephrons as varying, not only in certain anatomic characteristics which include position, size and length, but in functional terms as well. Some tubules, for example, might reabsorb a greater fraction of filtered sodium than others. Hence, it is conceivable that under different circumstances the total rate of filtration from all 2,000,000 glomeruli may remain unchanged, while the fraction of the total filtration in any particular group of nephrons may increase or decrease. In this fashion, a change in the rate of excretion of sodium may be effected without a change in the total filtered load.

Still another renal hemodynamic alteration that might conceivably influence the excretion of sodium to some degree has recently been re-emphasized by Vander, Malvin, Wilde, and Sullivan.[20] They raised the question of whether the reabsorption of sodium in the proximal tubule may be passive rather than active. They suggest that the colloid osmotic pressure of the blood in the peritubular vessels promotes a redisposition of water from the proximal tubular lumen into the vascular channels. This redisposition would tend to increase the luminal concentration of sodium and to promote its passive diffusion from the tubule. According to this concept, a change in the filtration fraction might alter sodium reabsorption because of changes in the colloid osmotic pressure of the blood in the peritu-

bular vessels. This theory has been criticized on several grounds: (1) it has been pointed out that it is unlikely that the colloids could influence the distribution of water across such a highly discriminating membrane as the proximal tubular epithelium; (2) it could not account for the bulk of the proximal tubular sodium reabsorption; (3) there is evidence that sodium does move against a gradient and, hence, must be transported by an active process.

The first criticism must be evaluated by experiment. The fact that it cannot account for the bulk of sodium reabsorption and the fact that there is undoubtedly active transport do not exclude the possibility that this transport might be facilitated in part by this mechanism. Thus, it is clear that altered rates of excretion of salt could be accounted for by small, and what are frequently referred to as "insignificant," changes in filtration rate, by a redistribution of filtration among the nephrons with no net change in the total rate of filtration, and, conceivably, by disproportionate changes in renal plasma flow and filtration rate.

This is a most difficult area to study, but the imposing nature of the experimental challenge does not reduce the significance of these possible physiologic regulatory devices. Furthermore, they might very well be responsive to direct consequences of alterations in the volume of the blood per se, and may represent rather automatic adjustments within the renal circulation itself.

There are certainly many studies wherein it is clear that a reduction of the volume of the body fluids decreases, and an expansion of the body fluids augments, the rate of excretion of salt. Changes in the disposition of volume without alteration in its total quantity also promote natriuresis and antinatriuresis. The passive erect posture, sitting, cuffing of the extremities, obstruction of the venous return from either the inferior or the superior vena cava, and opening an arteriovenous fistula all promote a diminished excretion of salt. In contrast, the relief of these obstructions and the assumption of a comfortable supine position augment the elimination of salt.[5-9, 21]

Let us deviate for the moment, to consider *which* volume may be undergoing the important change. An increase in the total volume of body water, an increase in the extracellular volume as a whole, an increase in the vascular volume, and an increase specifically in the arterial volume per se all promote an increased excretion of salt.

Several recent reviewers have stated[6, 7] that the infusion of iso-oncotic solutions of albumin does not promote the excretion of salt. However, the data from the experiments quoted did, in fact, demonstrate some increase in the excretion of salt when iso-oncotic solutions of albumin in saline were infused.[22] It was also apparent that this natriuresis was no greater than that achieved with the infusion of an equal volume of saline. Since the common denominator of these 2 types of infusion was an expansion of the intravascular volume, it is not illogical to assert that the expansion of the vascular volume was at least 1 determinant of the increased salt excretion.

This does not, of course, exclude the influence of alterations in the volume of the interstitial space. The experiments which suggest that the volume of this latter compartment may be important are those in which hyperoncotic solutions of albumin were found to expand the vascular volume (at the expense of the interstitial volume) and to diminish the rate of salt excretion.[23, 24] It may be that contraction of the interstitial fluid volume was important, but it is also possible that an increase in the colloid osmotic pressure of the plasma in the peritubular vessels was the important alteration.

Strauss and his co-workers[25] recently reported a study which emphasizes the amazing sensitivity of some receptors to the infusion of a salt solution. Subjects were prepared on a salt-free regimen, so that they had eliminated a "surfeit" of salt, and were excreting small quantities of it. They were then studied in a supine position, and at a time of day when a spontaneous, diurnal increase in the renal excretion of salt would not be expected. After

stable control periods, the subjects were infused with a small volume (150 ml.) of a 0 millimolar solution of saline over a period of 15 minutes. There was a prompt and unequivocal increase in the rate of excretion of sodium. The clearance of endogenous creatinine was said not to have changed significantly, and, since the infusate was hypotonic, it could hardly have increased the concentration of sodium in the filtrate.

Since this experimental design appeared to be uniquely sensitive to alterations in volume, Hollander[26] has been utilizing this protocol to compare the effects of the infusion of solutions which are identical in volume and composition with the exception that in half of the experiments, the infusate contains albumin at an iso-oncotic level. Thus far, there are 10 experiments, in which 150 ml. has been infused over a period of 30 minutes. Half of these have contained albumin. There is a small natriuretic response to both infusions, and although the mean increment in salt excretion is greater with the infusions which contain albumin, the significance of this difference is dubious. The question of a discriminating response to larger volumes is under study.

These experiments have some other interesting features. In 3 studies, it has been noted that the infusion of a volume as small as 25 ml. in 30 minutes, that is, less than 1 ml. per minute, will elicit a response which is identical to that noted after the infusion of 150 ml. It is difficult to imagine a central volume receptor sensitive to such minute changes, and the question must be raised whether the stimulation of a peripheral vein or the undetected influence of some other part of the experimental procedure may not be more important than the volume expansion itself. The experiment utilizing a mock infusion has not yet been done.

The characteristics of the redisposition of volume accomplished by changes in posture, cuffing of the limbs and obstruction of the vena cava, have suggested to several authors that volume receptors might exist in the upper half of the body. Some attention was drawn to the head itself by experiments that suggested that the application of a cuff around the neck might modify salt excretion.[27] However, this has not been confirmed by others.[28, 29]

Stretch receptors and baroreceptors have been known to exist in various parts of the circulatory system,[30] and recently considerable attention has been directed to those within the thorax. It had been noted by Gauer, Henry, Sieker, and Wendt[10] that negative pressure breathing promoted an increase in urine flow in dogs that began after about 10 minutes and reached its peak in 30 to 50 minutes. In contrast, Drury, Henry and Goodman[31] had reported that positive pressure breathing promoted a decrease in urine flow. These observations have been confirmed in man,[11, 17] and it seems reasonably clear that this is an effect on the excretion of water and that there is no primary effect on the rate of excretion of sodium or other solutes. The response can be blocked with vasopressin[15, 16] and there is no increase in urine flow while the subject is under a maximum water diuresis and the secretion of antidiuretic hormone is presumably completely suppressed.[15] The antidiuretic response to positive pressure breathing can be partially or completely inhibited with alcohol.[16] Surtshin and his collaborators[12] have demonstrated that the diuresis which occurs during negative pressure breathing is not significantly affected by renal denervation. Thus, the efferent arm of this reflex seems to be a diminished supply of antidiuretic hormone rather than some peripheral neural component.

Henry and his co-workers[13, 14] demonstrated that distention of the left atrium with a balloon resulted in a pronounced diuresis, and that the response to negative pressure breathing was either abolished or reduced by section of, or the application of cold to, the vagus nerves. They also recorded neural discharges from the vagus nerve, and by relating peak activity to events of the cardiac cycle, were led to conclude that the receptors probably respond to stretch rather than to pressure.

Henry and others[13] seemed to have excluded distention of the pulmonary arterial and ve-

nous system, except, perhaps, for that portion of the pulmonary vein that lies within the pericardium. Love and his collaborators[32] demonstrated that the amplitude of the pulsation of some part of the intrathoracic vascular system might be the important stimulus. They reported that pulsatile pressure breathing around a mean pressure of zero, or a positive pressure of 20 mm. Hg, promoted an increase in urine flow, whereas nonpulsatile positive pressure breathing did not.

In assessing the significance of this reflex, Henry, Gauer, and Sieker[33] reported that changes in blood volume from −30 per cent to +30 per cent influenced pressure concordantly throughout the circulatory system, and concluded that stretch receptors in the left atrium could therefore be influenced by changes in volume. However, it must be emphasized that this reflex only influences the excretion of water. Furthermore, Pearce[34] has reported that the integrity of the vagus nerve was not essential to the diuresis which resulted from iso-oncotic albumin infusion in dogs.

More recently, Barger and his collaborators[35, 36] have concerned themselves with another area of the vascular tree which may be implicated as a volume receptor. They noted that a decrease in blood pressure in the carotid sinus is accompanied by a decreased rate of excretion of sodium. They suggest that the retention of sodium may be due to the release of norepinephrine as blood pressure in the carotid sinus decreases. This hypothesis remains to be reconciled with the observations of Pearce[34] that denervation of the carotid sinus does not interfere with the diuresis which follows the infusion of iso-oncotic solution of albumin in the dog.

If volume receptors are of importance in the regulation of salt excretion, the efferent arc would presumably be 1 or more of the following: a neural impulse, the diminished secretion of a salt-retaining hormone, or the secretion of some humoral agent which was primarily concerned with increasing the excretion of salt, i.e., a natriuretic hormone.

The role of the central nervous system in the regulation of salt excretion continues to be enigmatic. There is a considerable distribution of neural elements to the kidneys, but there is no convincing evidence that any of these fibers terminate within renal tubular cells.[37] The renal nerves appear to be involved primarily with the vascular system. On the other hand, there are both patients and experimental animals in whom lesions of the central and peripheral nervous system may be accompanied by an increased excretion of salt in the urine.

Schwartz and his co-workers[38] have explained "salt wasting" in 1 group of patients as being due to an inappropriate secretion of the antidiuretic hormone, the retention of water, expansion of body fluid volume, and a consequent augmented rate of excretion of salt. These patients, and others who appear to represent the same renal disorder, have had central nervous system lesions. The experimental denervation diuresis provoked by section of the splanchnic nerves may be due solely to an influence on renal vessels and altered hemodynamics.[6, 9] Jungmann and Meyer[39] and, more recently, Wise[40] have provoked an increased renal excretion of salt by producing lesions in the brain stem. Finally, some patients have been seen with coexistent central nervous system lesions of recent origin, hyponatremia and dehydration; in this group, which has been designated "cerebral salt wasters," it seems virtually impossible to implicate the administration, and the retention, of water.[9] The influence of the central nervous system on the kidney remains to be elucidated. Again, it should be stated that the denervated kidney can still respond in a normal fashion to stimuli which ordinarily promote the excretion or retention of salt.

The most recent phase of the development of this problem concerns the influence of volume on the secretion of the hormone, aldosterone. Dr. Farrell and Dr. Bartter will undoubtedly concern themselves with this aspect, so I shall not dwell on this problem in any detail. However, assuming that changes in volume are somehow appreciated by a receptor mechanism and translated into an altered rate of secretion of aldosterone, we must still

raise the question of the significance of this hormone in the regulation of the excretion of salt.

There is no doubt that aldosterone does have a profound influence on the renal tubular reabsorption of salt.[9] However, its effects cannot always be demonstrated, and when they do occur, they appear only after a lapse of time.[41] The prompt, acute alterations in the excretion of salt in response to absolute changes in blood volume or to redisposition of volume can occur in the adrenal-insufficient animal and human.

In summary, there are undoubtedly many mechanisms that influence the excretion or retention of salt, and thereby control the volume of body fluids within narrow limits. As of this time, one might list them as follows:

1. Basic mechanisms, which are intrinsic to the kidney itself, which are determined by filtered load, distribution of this load among various nephrons, and perhaps changes in filtration fraction. These mechanisms are most likely responsible for the acute adjustments, and it is probable that the volume of the blood influences these determinants by minor variations in blood flow and perfusion pressure. Furthermore, it is difficult in many circumstances to prove that these minor changes were not responsible for an observed alteration in the excretion of salt.

2. Neural regulators which probably express themselves in fine hemodynamic adjustments. The possibility of some other influence is not resolved.

3. Hormonal influences which, in turn, may be responsive either to changes in volume or to some function thereof. The evidence that volume influences antidiuretic hormone secretion has been presented. The probability that volume influences the secretion of aldosterone will be considered in a subsequent paper.

4. The existence of a natriuretic hormone is highly speculative. It should be and is being sought. Until it is found, it does not seem profitable to speculate further on how its secretion may be altered.

Throughout this discussion, I have not meant by any means to underestimate the importance or the significance of mechanisms that have an extrarenal origin. However, the *certain* exclusion of renal hemodynamic influences is not easy and must always be considered. Perhaps new and better tools must be applied to these problems.

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Adrenoglomerulotropin

By GORDON FARRELL, M.D.

The secretion of aldosterone by the zona glomerulosa of the adrenal cortex is controlled by a system which is not completely understood. Volume receptors are of importance although other receptors may be involved. The midbrain contains elements which influence the secretion of the steroid since lesions in this area of the brain stem reduce aldosterone output. A lipid factor, adrenoglomerulotropin, obtained from pineal extracts, selectively stimulates secretion of aldosterone; but a second factor, also from the pineal gland inhibits the output of aldosterone as well as of cortisol. It is suggested that the control of the secretion of aldosterone may be by an excitatory-inhibitory system involving the interplay of pituitary corticotropin, adrenoglomerulotropin and an anticorticotropin of pineal origin.

THREE questions are frequently asked about aldosterone. First, where is aldosterone synthesized; second, in what way is its secretion controlled; and finally, what is the role of the steroid in certain diseases? Considerable information has been brought to bear on these points during the relatively few years since aldosterone was discovered. Perhaps we can summarize some of the more recent developments.

With regard to the first question, almost everyone is agreed, now, that aldosterone is secreted by the outermost zone of the adrenal cortex, the zona glomerulosa. This zone undergoes selective hypertrophy in response to deprivation of sodium at a time when the secretion of aldosterone is markedly enhanced. This is illustrated in figure 1, from a recent experiment. Figure 1*A* is a photomicrograph of the adrenal gland of a dog which was given normal amounts of sodium. Figure 1*B* shows the effects of a 3-month period of deprivation of sodium. The rate of secretion of aldosterone in dogs in which the sodium intake is low (2 mEq./day) is approximately 3 times that in animals on a normal diet. It is of interest that the output of cortisol is frequently lower in dogs which are deprived of sodium than in control animals, indicating that the stimulation of the secretion of aldosterone is not mediated by an increased release of ACTH.

Additional and very convincing evidence that the zona glomerulosa is the locus of synthesis of aldosterone has recently been provided by the elegant experiments of Ayres, Gould, Simpson, and Tait,[1] and of Stachenko and Giroud.[2] Zona glomerulosa tissue synthesizes aldosterone in vitro, whereas very little is elaborated by tissue from the inner zones. Interestingly enough, ACTH (which stimulates steroidogenesis when added to incubates of tissue from the zona fasciculata and the zona reticularis) does not appear to affect the zona glomerulosa in vitro.[2] Taken together, these observations appear to settle the long dispute touched off by the original publications of Deane and Greep some years before aldosterone was discovered. These authors postulated that the zona glomerulosa secretes an electrolyte-active steroid independently of the rest of the adrenal gland,[3, 4] and their speculations have apparently been proven correct.

The second question, with regard to the *modus operandi* of the system for the control of the secretion of aldosterone, is not so readily answered. The stimuli which alter aldosterone output are many.[5] However, alterations in the balance of sodium and in the volume of body fluids stand out as especially important. The restriction of sodium is a

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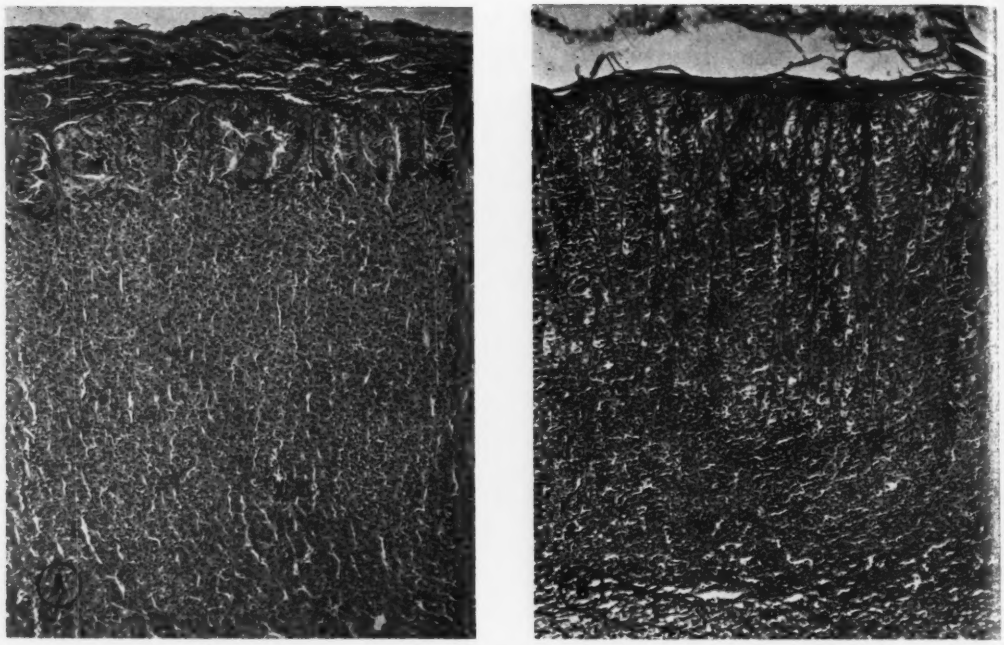

Figure 1

Adrenal gland of the dog. A. *Control. From above downward are zona glomerulosa, zona fasciculata and zona reticularis.* B. *Adrenal gland of a dog on a low-sodium diet for 3 months. Note the hypertrophy of the zona glomerulosa.*

constant and potent stimulator of the secretion of aldosterone in all species which have been studied, although the manner in which the organism detects the depletion or repletion of sodium is still unknown. Sodium-sensitive chemoreceptors somewhere within the vascular system are a possibility, but thus far none have been described. The subject of volume receptors for the control of the secretion of aldosterone has received considerably more study. The location of the receptors is still in question; the atria may very well contain them as well as the carotid arteries.[6, 7] This subject will be considered by subsequent speakers in this symposium.

Whatever the nature of the receptor systems may be, it seems clear that an *effector system* must exist by which the zona glomerulosa is stimulated to increase the secretion of aldosterone in response to changes in the internal environment. It is this effector system which I should like to consider briefly now. The participation of nerve fibers can probably be discounted since denervation of the adrenal glands has no effect on the output of aldosterone.[8] Direct action on the gland by electrolytes of the blood has been suggested by some authors as a possible stimulus,[9] but the modest effects on steroidogenesis induced by alterations in the sodium and potassium content of the adrenal perfusate are probably too small to account for the changes in aldosterone output observed under various experimental conditions. Furthermore, in many instances, profound changes in the rate of secretion of the steroid occur without any change in the concentrations of electrolytes in the blood.[5]

The possibility that the zona glomerulosa is subject to the influence of a tropic factor which is specific for this zone was entertained in our laboratory a few years ago, and indirect

Table 1

Steroidogenic Properties of Extracts of Beef Diencephalon or Pineal

Experiment	No. of experiments	Aldosterone μg./100 Kg. body wt./hr.	Cortisol μg./Kg. body wt./hr.
Intact controls	9	31.2 ±3.7	32.8 ±3.9*
Decerebrate controls†	8	7.86±0.87	2.19±0.49
Decerebrate controls	6	8.5 ±0.95	1.72±0.38
Decerebrate, infused with saline extracts of whole diencephalon, including pineal	7	24.21±3.2‡	1.93±0.55
Decerebrate, infused with saline extracts of pineal alone	6	30.3 ±2.9‡	2.17±0.51
Decerebrate, infused with acetone extracts of pineal	4	25.0 ±4.15‡	3.98±1.03
Decerebrate, infused with hexane fraction from water-hexane partitioning of residue from acetone extracts	4	34.6 ±4.2‡	3.69±0.66
Decerebrate, infused with aqueous fraction from water-hexane partitioning	3	10.92±3.2	9.93±3.0

*Mean and standard error.

†Animals used 4 to 6 hours postdecerebration.

‡Statistically significant ($P < .01$) when compared with the corresponding controls.

evidence for such a factor was obtained.[10] The concept has since received support from other laboratories.[11] Although the existence of this hormone was originally proposed on the basis of admittedly indirect evidence, we feel that presently available data definitely establish that the hormone exists, and that it can be obtained from extracts of the diencephalon. This hormone was first demonstrated in the course of assays of neutral saline extracts of various areas of beef diencephalon.[12] The assay animal was the midcollicular, decerebrate dog in which the brain above the level of the cut was removed together with the pituitary and pineal glands. The concentrations of steroids were determined by analysis of adrenal venous blood which was collected during the infusion of test solutions. Extracts of whole diencephalon stimulated the secretion of aldosterone (table 1). When the diencephalon was divided into anterior and posterior parts, activity was found primarily in the posterior portion when this structure included the pineal gland. When the pineal gland was removed and assayed separately, most of the activity was found to be present in the pineal gland (table 1). An acetone extract of pineal glands also yielded activity.[13] To our surprise, when the residue from the acetone extract of pineal glands was partitioned between hexane and water, the activity was recovered from the hexane fraction indicating a definite degree of lipid solubility (table 1). We originally proposed the name *glomerulotropin* for this hormone, but reservations of renal physiologists who feel that the name implies an effect on the glomerulus of the kidney lead us to suggest that it be called *adrenoglomerulotropin.*

We have recently carried out a number of procedures designed to purify adrenoglomerulotropin. Chromatography of the residue from acetone or alcohol extracts of pineal gland on a florosil column, using hexane as the mobile phase, results in a many-fold purification. The active fractions from florosil columns have been subjected to paper chromatography. In the system propylene glycol–hexane, the activity runs with or just behind the solvent front, indicating a polarity much less than that of any of the corticosteroids. In the system water-chloroform-ethanol (3:5:5) on silicone-treated paper, in which the developing phase is aqueous ethanol, the active material runs

Table 2

A Factor from Pineal Extracts Which Inhibits Steroid Secretion

Experiment	No. of experiments	Aldosterone μg./100 Kg. body wt./hr.	Cortisol μg./Kg. body wt./hr.
Intact animals, infused with vehicle (saline) or extract of cerebral cortex	8	36.0 ±3.2*	35.9 ±3.3
Intact animals, infused with fraction I from pineal	5	15.8 ±1.5 ($P < .001$)	24.6 ±2.7 ($P < .05$)
Decerebrate animals, infused with vehicle†	5	15.1 ±1.5	
Decerebrate, infused with fraction I from pineal	8	6.8 ±0.4 ($P < .001$)	
Decerebrate, infused with adrenoglomerulotropin fraction from pineal	5	29.3 ±3.0	

*Mean and standard error.

†Animals used for assay 3 hours after decerebration. The rate of secretion of aldosterone was thus somewhat higher than in dogs permitted to rest for longer periods postoperatively, cf., table 1, experimental groups 2, 3.

with an R. F.* of about 0.9, suggesting the presence of 1 or more groups contributing some degree of water solubility. The active fractions from paper chromatograms contain very small amounts of solid material. Although precise quantitation is not possible, we can say that an effective dose infused into an assay dog over a 2-hour period is certainly less than 100 μg. Thus far we have not succeeded in isolating sufficient quantities of the material to permit chemical characterization. The substance does not appear to have a specific ultraviolet absorption curve, at least not in the amounts isolated. A weak reaction for phenols is given in the active region of the chromatograms; however, we have no assurance that this reaction is not due to a contaminant. Further characterization obviously must await the preparation of larger amounts of the hormone.

In the course of our efforts to purify adrenoglomerulotropin, we had noted that the potency appeared to increase as fractions containing adrenoglomerulotropin were purified on column or paper chromatograms. We wondered whether the crude extracts contained an inhibitor which was later separated from adrenoglomerulotropin. In screening some of our recent chromatograms with bio-assay, we found that eluates of 1 region of the chromatograms (fraction I, table 2), when infused into the decerebrate assay dog, did indeed reduce the secretion of the steroid below the basal level. This eluate has also been assayed for inhibitory activity in *intact* dogs. Solutions of the suspected fraction were administered intravenously 1 hour prior to cannulation of the adrenal vein and during a 2-hour period of collection of adrenal venous blood. There appears to be no question that the material actively inhibits steroidogenesis (table 2). It is readily separated from adrenoglomerulotropin on chromatograms since it has an R. F. of about 0.3 in the system water-chloroform-ethanol (3:5:5) on silicone-treated paper.

The inhibitor appears to be of pineal origin since extracts of equal amounts of cerebral cortex do not inhibit steroidogenesis. Its chemical nature is also unknown except for the fact that it appears to be a lipid. Curiously enough, although it inhibits the secretion of cortisol (table 2), the reduction in the secretion of aldosterone is even more striking. Although interpretation of these results must be tempered with caution, one is tempted to

*R.F.=the rate of movement of the band of the substance under scrutiny divided by the rate of movement of the advancing front of the liquid phase.

Table 3

Steroid Secretion Rates in Pinealectomized Dogs

Experiment	No. of animals	Aldosterone μg./100 Kg. body wt./hr.	Cortisol μg./Kg. body wt./hr.
Intact animals	9	31.2 ±3.7*	32.8 ±3.9
4 hours postoperative			
sham	3	36.5 ±8.5	34.9 ±4.8
pinealectomy	3	14.3 ±1.42	31.2 ±2.5
1 week postoperative			
sham	5	34.6 ±5.7	39.3 ±8.1
pinealectomy	6	36.8 ±4.2	54.4 ±4.5
3 weeks postoperative			
sham	4	37.9 ±4.6	38.9 ±3.9
pinealectomy	8	24.1 ±2.9	39.4 ±3.1
16 weeks postoperative (last 12 weeks on low-sodium diet)			
sham	2	88, 105	13.3, 16
pinealectomy	2	193, 158	30.8, 33.4

*Mean and standard error.

speculate that the inhibitor is a normal secretory product of the pineal gland and that it participates in the regulation of steroidogenesis. The material (which may be thought of as an *anticorticotropin*) may compete with pituitary corticotropin and with adrenoglomerulotropin to restrain adrenal activity. The quantitative relationship between the 3 factors in any given situation may determine the final rate of steroid secretion. I should like to emphasize that this is, at best, a working hypothesis and only with time and additional study will it be possible to evaluate the relative biologic importance of these materials in the regulation of the secretion of aldosterone and cortisol.

The isolation from the epiphysis cerebri of factors which play a part in the control of the secretion of steroids naturally raises the question of the consequences of pinealectomy. Since adrenoglomerulotropin is found in pineal extracts, one might imagine that removal of the gland would result in cessation of the secretion of aldosterone. This is not the case. It is true that in the first few hours following pinealectomy, a decrease in the secretion of aldosterone was observed (table 3).[14] However, 1 week postoperatively, the secretion rate of the steroid was found to be at control levels; moreover, even though the rate of secretion was slightly lower 3 weeks postoperatively than it was 1 week after operation, it is apparent that the pinealectomized dog is quite capable of synthesizing aldosterone. Pinealectomized dogs also respond, in a perfectly normal fashion, to deprivation of sodium, showing hypertrophy of the zona glomerulosa and a markedly increased output of aldosterone. Indeed, in 2 pinealectomized dogs recently studied on a low-sodium regimen for a period of 3 months, the rate of secretion of aldosterone (as well as of cortisol) was found to be nearly twice that in sham-operated dogs on the same diet (table 3). It would appear, at least in this experimental situation, that the long-term consequences of pinealectomy are those which might be anticipated from removal of inhibitory, rather than stimulatory, influences.

Some of the confusion which seems to confront the investigator at this stage of the problem may be related to our tendency to oversimplify. It is quite possible that we are dealing not with a single gland but with 2 glands. Another glandular structure in this area of the brain is the subcommissural organ, a structure which, though known for many years, has only recently attracted the attention of physiologists. Information on the functions of the subcommissural organ is still very limited. However, there is evidence that it is important in the control of water bal-

ance.[15] Taylor, in our laboratory, has been able to relate the subcommissural gland, or the area around it, to the control of the secretion of aldosterone. Taylor placed high frequency coagulation lesions in various areas of the brain stem in cats and determined, by analyses of adrenal venous blood, the rates of secretion of aldosterone. She found that lesions involving the central gray substance of the midbrain, at the level where the cerebral aqueduct opens anteriorly into the third ventricle, reduce the output of aldosterone. Effective lesions need not involve the pineal gland, but the subcommissural organ was disrupted in most of the experiments in which a decreased output of aldosterone was found, suggesting that this structure might be the source of stimulatory influences. On the other hand, it is possible that the effective lesions interrupt nerve pathways destined for other areas of the brain which are concerned with steroid regulation. It is anticipated that studies currently under way will permit a decision on this point.

I think it should be clear from the data which we have presented that there are many more questions about the regulation of the secretion of aldosterone than there are answers. One topic, mentioned at the beginning, remains for discussion: the possible role of abnormalities in the secretion of aldosterone in various diseases. Increased output of aldosterone has been implicated in a number of diseases, including cardiac failure, nephrosis, cirrhosis, and possibly essential hypertension. In only 1 syndrome, however, has a clear-cut relationship been established between the secretion of the steroid and the development of a clinical disorder. This is, of course, in the Conn syndrome, in which an abnormally high rate of secretion of aldosterone by an adrenal adenoma or by a hyperplastic adrenal gland is clearly the cause for the hypertension, the abnormal concentrations of electrolytes in the blood, and the renal pathology. Whether hypersecretion of the steroid complicates or plays an etiologic role in other diseases must, at this time, be considered an open question. An important recent discovery made in the laboratory of Sayers should probably be mentioned at this time. Sayers and his collaborators, using the isolated heart-lung preparation of the rat, have been able to show that aldosterone has a direct digitalis-like action on the myocardium.[17] This is shown especially well in increased work capacity of the heart-lung preparation from adrenalectomized animals. This observation raises the question of whether aldosterone may serve to support myocardial function in stressful situations. If this proves to be the case, the increased secretion of aldosterone in certain diseases may represent one of the means by which the organism attempts to compensate for the cardiovascular consequences of the disease.

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Fragments of Life

About the middle of the last century the younger physiologists broke away from the vitalistic traditions which had been handed down to them, and set about to investigate living organisms piece by piece, precisely as they would investigate the working of a complex mechanism. This method seemed to them to promise success, and was popularized by such masters of clear and forceful expression as Huxley. It is still the orthodox method of physiology, but the old confidence in it has steadily diminished in proportion as exact experimental investigation has shown that the various activities of a living organism cannot be interpreted in isolation from one another, since organic regulation dominates them.—J. S. Haldane. *Respiration*. New Haven, Yale University Press, 1922, p. 32.

On the Hemodynamic Regulation of the Secretion of Aldosterone

By FREDERIC C. BARTTER, M.D., AND DONALD S. GANN, M.D.

Evidence is presented that the secretion of aldosterone in dogs may be considerably influenced by hemodynamic factors. The experiments indicate that secretion of aldosterone may be decreased by expanding the "low pressure" system, and may be increased by reducing the amplitude of the pulse pressure in the carotid arteries. The decrease may be prevented by prior section of the vagi, the increase by prior denervation of the thyro-carotid arterial junctions.

PREVIOUS experiments have pointed to some function of blood volume as a major factor in the control of the secretion of aldosterone.[1] In the present studies, we have proceeded on the assumption that the secretion of aldosterone involves a neurohumoral final common pathway, with a mechanism within the central nervous system which integrates signals generated by hemodynamic stimuli. The experiments described here are attempts to delineate the afferent pathways of these stimuli and the essential nature of the hemodynamic stimuli themselves. Some of the work discussed here has been presented elsewhere.[2-5]

The experiments were performed on healthy mongrel dogs which had been anesthetized with sodium pentobarbital. Adrenal venous blood was collected intermittently by the method of Hume and Nelson.[6] The secretion rate of aldosterone was determined either by Mills' modification[2] of the method of Neher and Wettstein[7] or by the method of Kliman and Peterson.[8] Constriction of the thoracic inferior vena cava (caval constriction) was effected through a closed chest by means of an inflatable cuff passed about the vessel and led out through the thoracic incision. Bilateral constriction of the common carotid arteries was effected by means of silk strings passed about the carotid arteries and led through double-lumen plastic cannulas, held rigid to prevent pull on the arteries. Pressures were measured in the brachial and lingual arteries, right atrium, femoral vein and esophagus by means of Statham pressure transducers and recorded continuously on a Sanborn 8-channel recorder. All acute experiments were designed so that a control collection of adrenal blood for measuring the concentration of

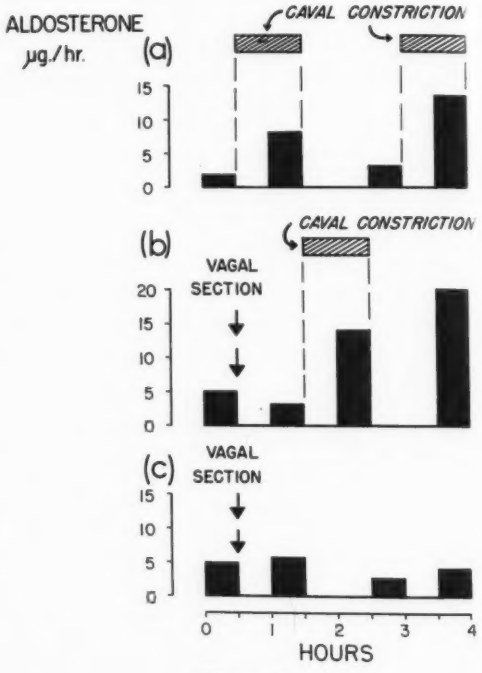

Figure 1

Effect on secretion of aldosterone of: (a) *constriction and release of inferior vena cava;* (b) *the same after vagotomy; and* (c) *vagotomy alone. (Republished by permission of Science.[2])*

From the Section on Clinical Endocrinology, National Heart Institute, National Institutes of Health, Bethesda, Md.

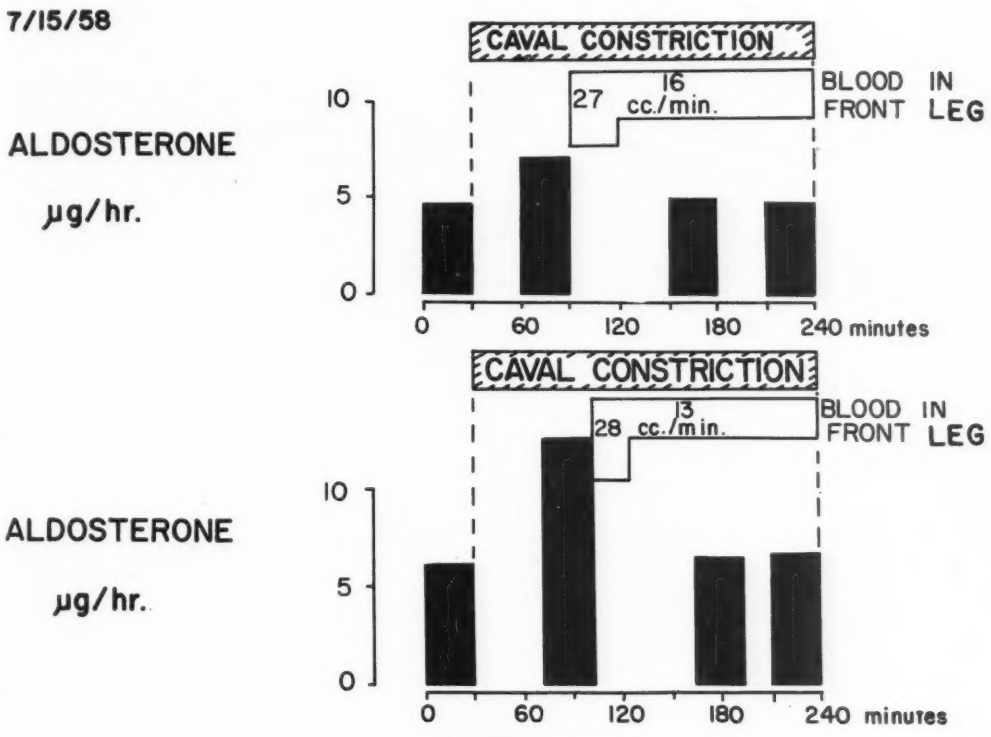

Figure 2

Experiments on 2 dogs showing reversal of the effect of caval constriction on secretion of aldosterone by rapid infusion of blood above the constriction.

aldosterone was obtained before application of the specific stimulus. Blood lost, or withdrawn for assay, was replaced by homologous transfusion.

Constriction of the supradiaphragmatic inferior vena cava traps blood "below" the constriction, with a resultant depletion of blood "above" the constriction. Such a constriction is consistently effective in increasing the secretion of aldosterone; this effect can be measured, reproducibly, within 1 hour of the application of the constriction. Upon release of the constriction, there is, also reproducibly, a fall in the secretion of aldosterone which occurs within an hour and a half.[2] The constriction can then be reapplied and, within an hour, there is again an increase in the secretion of aldosterone. An experiment of this type is illustrated in figure 1*a*.

It has been suggested by Davis and coworkers[9] that caval constriction stimulates an increase in the secretion of aldosterone in some way other than by modifying a function of the blood volume. In their experiments, the increase in the secretion of aldosterone, which ordinarily follows caval constriction, could not be prevented by infusion of a quantity of dextran which was calculated to compensate for the blood sequestered or lost by application of the constriction. However, the extent of hemodynamic changes evidently depends on the degree of the constriction, and the susceptibility of these changes to reversal by infusion depends upon the rate of infusion as well as upon its volume. Figure 2 shows 2 experiments in which an increase in the secretion of aldosterone was first produced by caval constriction, and the effect then nullified while

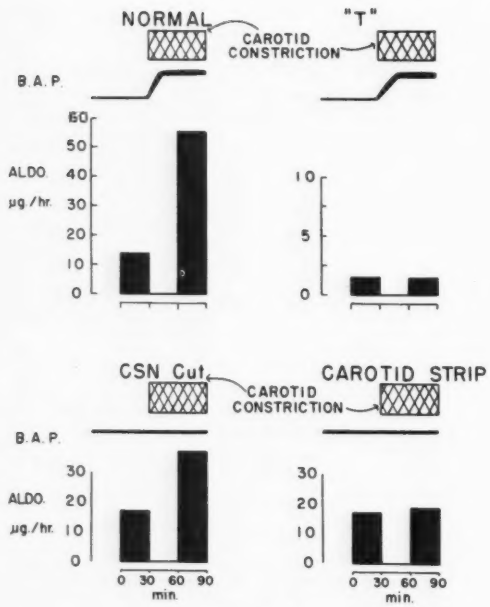

Figure 3

Effect of carotid constriction on brachial arterial pressure (B.A.P.) *and secretion of aldosterone in dogs.* Top left. *Normal.* Bottom left. *Carotid sinus denervation* (CSN cut). Top right. *Thyro-carotid arterial junction denervation* ("T"). Bottom right. *Carotid sinus and thyro-carotid arterial junction denervation* (carotid strip).

the constriction was maintained, by rapid infusion of blood above the constriction. This evidence suggests that caval constriction is, in fact, a hemodynamic stimulus.

Bilateral section of the vagus nerves in the neck does not prevent the *rise* in the secretion of aldosterone which follows caval constriction, but it does prevent the *fall* which follows release of the constriction.[2] An experiment of this type is shown in figure 1*b*. As shown in figure 1*c*,[2] vagotomy per se has no effect upon the secretion of aldosterone. Both the increase in the secretion of aldosterone which follows caval constriction despite vagotomy, and the absence of the fall in the secretion of aldosterone in the vagotomized animal after the constriction is released are reproducible phenomena. This evidence is consistent with the hypothesis that impulses leading to decreases in the secretion of aldosterone may arise in the atria or great vessels and be conveyed to the central nervous system via the vagus nerves. It also suggests that stimuli leading to increases in the secretion of aldosterone are mediated by some other pathway.

Bilateral constriction of the common carotid arteries low in the neck (carotid constriction) produces an increase in the rate of secretion of aldosterone. In the intact animal, it also gives rise to an increase in systemic (extracarotid) arterial blood pressure. Prior stripping of the carotid arteries of their nerve supply—from above the carotid sinuses to low in the neck—abolished the increased secretion of aldosterone and the increase in blood pressure which otherwise follow carotid constriction.

It is possible to dissociate the effects of constriction of the common carotid artery on the secretion of aldosterone from the effects on the blood pressure. Some animals were subjected to a careful denervation of the carotid sinuses alone. In these animals carotid constriction produced normal increases in the secretion of aldosterone even though the usual pressor responses were absent. In another group of dogs, the thyro-carotid arterial junctions were denervated without denervation of the carotid sinuses or of the remainder of the carotid arteries; the region of the thyro-carotid arterial junctions has been shown to be a baroceptor area in both the dog[5] and the cat.[10] In these dogs, carotid constriction produced no increases in the secretion of aldosterone despite intact pressor responses.[3] The effects of carotid constriction on the peripheral arterial pressure and on the secretion of aldosterone are shown schematically in figure 3. Statistical evaluation of the data derived from these experiments reveals no significant difference between normal and sinus-denervated animals with respect to increases in the secretion of aldosterone following carotid constriction. However, the responses of the group with denervated thyro-carotid arterial junctions were significantly different ($p < .001$) from those of the normal group. Denervation

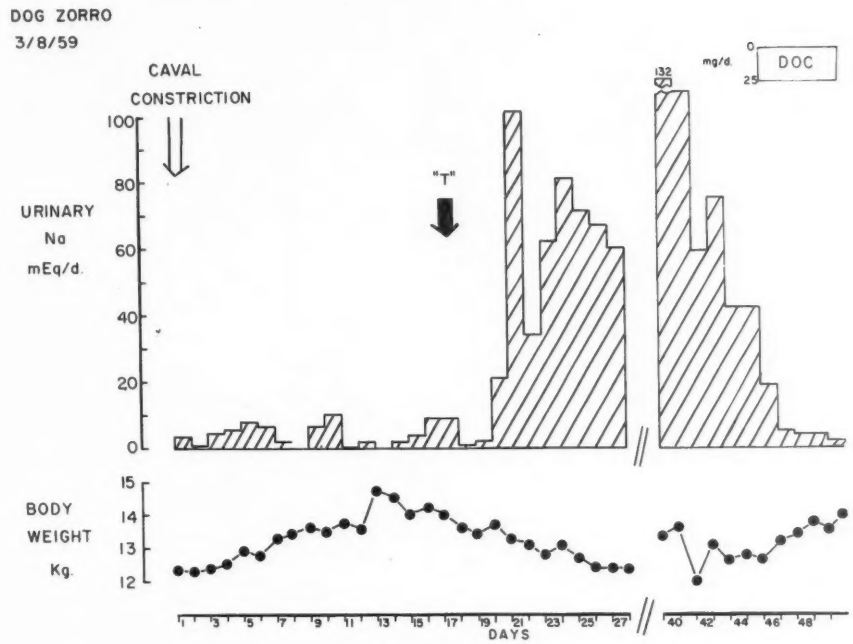

Figure 4

Effects of caval constriction, of denervation of the thyro-carotid arterial junctions ("T") and of administration of desoxycorticosterone (DOC) *on urinary sodium and body weight in a dog receiving a daily sodium intake of 50 mEq.*

of this region in another group of dogs was also shown to prevent the acute increase in the secretion of aldosterone which ordinarily follows caval constriction. Again, the results were highly significant statistically.[3] These data suggest that the increases in aldosterone secretion following caval or carotid constriction depend on the integrity of the nerves arising at the thyro-carotid arterial junction.

Chronic constriction of the supradiaphragmatic inferior vena cava commonly produces a form of secondary aldosteronism characterized by retention of sodium, ascites and, at times, edema.[11] The retention of fluid in this condition is an exceedingly complex phenomenon, and it seems clear that aldosterone plays only a part in its development or maintenance. An experiment on an animal prepared in this way is shown in figure 4. Following caval constriction, there was retention of sodium with gain in weight and the development of ascites on an intake of approximately 50 mEq. per day of sodium. This animal was then subjected to bilateral denervation of the thyro-carotid arterial junctions. This operation was followed by natriuresis, a loss of

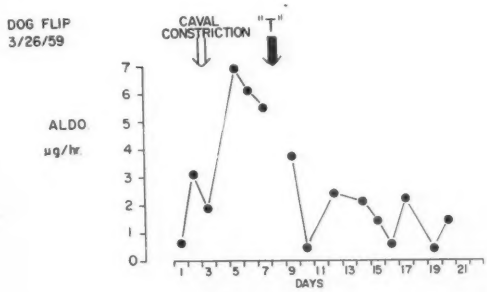

Figure 5

Effects of caval constriction and of denervation of the thyro-carotid arterial junctions ("T") on secretion of aldosterone in a dog with chronic adrenal cannula receiving a daily sodium intake of 60 mEq.

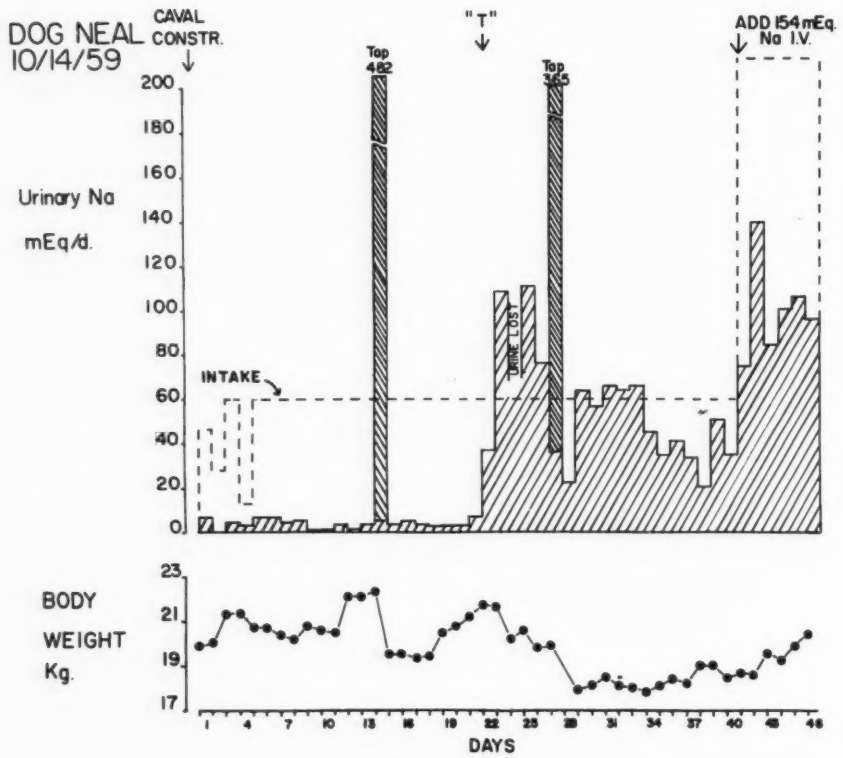

Figure 6

Effects of caval constriction, of denervation of the thyro-carotid arterial junctions ("T") and of increase of sodium intake on urinary sodium and body weight.

ascites and a reduction in weight. That this response was not due to development of collateral circulation is shown by the response to desoxycorticosterone, which produced retention of sodium and gain in weight. Figure 5 shows a series of values for the secretion of aldosterone in a dog with a chronic adrenal cannula. The animal was first subjected to caval constriction and, later, to denervation of the thyro-carotid arterial junctions. Caval constriction produced an increased secretion of aldosterone which was reversed after the denervation.

Because ascites per se may reduce the ability to retain further amounts of sodium,[12] still another preparation was utilized. Dogs with ascites following caval constriction, receiving a constant sodium intake of 60 mEq. per day, were subjected to paracentesis. After the abdominal fluid had reaccumulated, they were subjected to bilateral denervation of the thyro-carotid arterial junctions. In order to provide a maximal stimulus to sodium retention, a paracentesis was again done. One such experiment is shown in figure 6. It is seen that following the denervation, the ability of the animal to retain sodium on a daily intake of 60 mEq. was impaired. However, an increase in the intake of sodium to more than 200 mEq. per day resulted in retention of sodium and gain in weight, indicating that caval constriction was still present. The fact that this last phenomenon can be demonstrated in bilaterally adrenalectomized animals[13] is further evidence that the retention of sodium in the caval-constricted dog is not

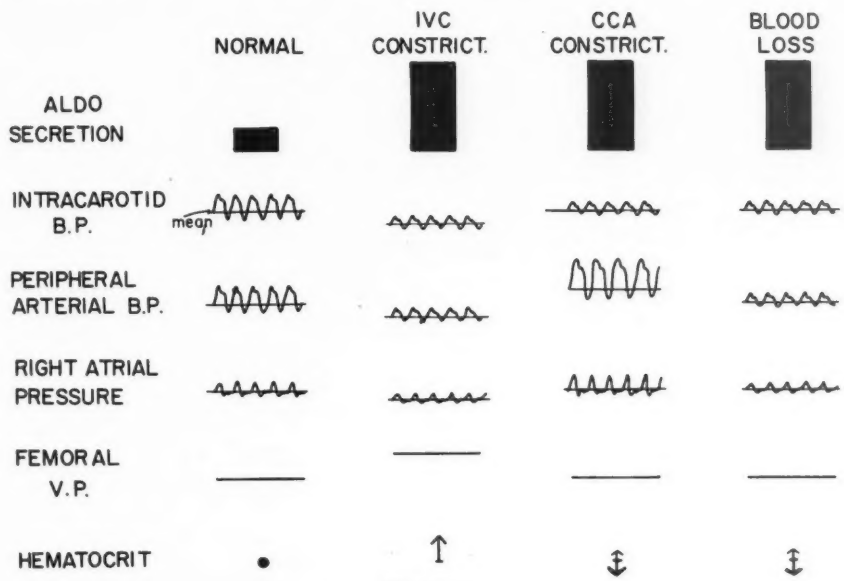

Figure 7

Schematic representation of the effects of constriction of the inferior vena cava (IVC), *of constriction of the common carotid arteries* (CCA) *and of blood loss on secretion of aldosterone and certain hemodynamic variables.*

entirely dependent on the increased secretion of aldosterone.

Hemodynamic data derived from acute experiments with caval and carotid constriction have been compared with data from animals which were subjected to blood loss,[4] a stimulus which is known to increase the secretion of aldosterone.[1, 14] Such data are summarized diagrammatically in figure 7. Of the changes measured, only diminished intracarotid pulse pressure was consistently associated with the increase in the secretion of aldosterone. Because of the reflex increase in mean systemic blood pressure as the carotid pulse pressure decreased,[15] it was possible to maintain, or even to increase, intracarotid mean pressure during carotid constriction by using a small amount of constriction.

It is not yet possible to assess the role of pulse pressure in the aldosteronism of cardiac failure in man: we are engaged in such studies at the present time. The published literature on man does contain data consistent with the schema suggested by the results in the dog. Eichna and associates, for example, induced loss of sodium and of weight in patients with "low-output" cardiac failure by rapid digitalization.[16] In each patient, arterial pulse pressure rose promptly with digitalization. In patients with "high-output" failure, the situation is more complex. If hypersecretion of aldosterone is an important element in the disease picture, it is clear that a low absolute value of pulse pressure cannot be a prerequisite, since elevated pulse pressure and edema may certainly coexist in beriberi heart disease. However, one of the earliest responses of patients with beriberi heart disease to thiamine is an *increase* of pulse pressure which precedes the loss of sodium and of weight.[17, 18] Thus, a relative decrease of pulse pressure may be related even to the aldosteronism of high-output failure.

In summary, figure 8 presents schematically a working hypothesis concerning the hemodynamic control of the secretion of aldosterone. A dual mechanism is illustrated. Secretion of aldosterone may be *inhibited* by

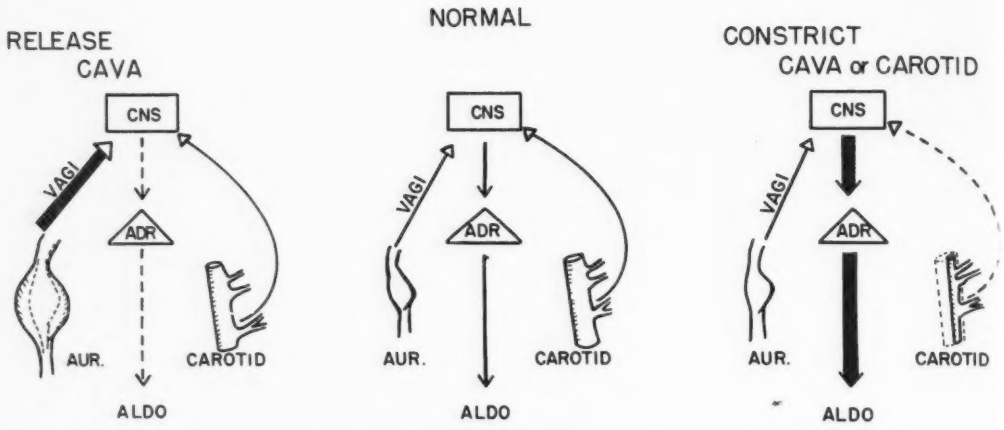

Figure 8

Schematic representation of a proposed dual mechanism mediating decreases (e.g., by release of caval constriction) or increases (e.g., by caval or carotid constriction) in secretion of aldosterone. Aur. = *right atrium and great vessels;* CNS = *central nervous system;* ADR = *adrenal gland;* Aldo = *aldosterone.*

expansion of the "low pressure" system; the afferent impulses traverse vagal pathways. Secretion of neurohormone may be *increased* by constriction of (that is, decrease of pulse pressure in) the common carotid artery; the afferent impulses arise from baroreceptors in the region of the thyro-carotid arterial junctions.[5]

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The Laws of Nature

It is one thing for the human mind to extract from the phenomena of nature the laws which it has itself put into them; it may be a far harder thing to extract laws over which it has no control. It is even possible that laws which have not their origin in the mind may be irrational, and we can never succeed in formulating them.—A. Eddington. *The Philosophy of Physical Science.* Ann Arbor, University of Michigan Press, 1948, p. 179.

Discussion

Dr. Hilton: Dr. Farrell, I was interested in the fact that some of the commercial pineal extracts have ACTH, as well as aldotropic, activity. You will pardon me if I use the word "aldotropic." As a former renal physiologist, I can't quite see "glomerulotropic."

Both of these extracts had ACTH, as well as aldotropic, activity. If I am correct, the acetone-soluble extract lost its ACTH activity while retaining its aldotropic activity. Have you treated extracts of the anterior pituitary similarly to see if, as indicated by some of our experiments, there is aldotropic activity? By treatment of the anterior hypophysis in a similar manner can one associate the 2 activities?

Dr. Farrell: I should like, first, to speak to the question of the name. The name, glomerulotropic, comes from the gentleman on my left, Dr. Sayers.

Chairman Sayers: Let me answer that. I will be very brief. The "tropin" can't stimulate the steroid. We assume that it stimulates the tissue, namely, the glomerulus. Hence, the name, glomerulotropic. In all deference to the renal physiologists here, why, we will certainly accept the name adrenoglomerulotropic. (Laughter)

Dr. Farrell: Do you accept it?

Dr. Hilton: For the moment.

Dr. Farrell: With regard to the presence of ACTH in commercial pineal extracts, I think that some of the difficulty may arise from incomplete separation of the pineal from the pituitary gland in the slaughterhouse. In 1 shipment of frozen beef *pineals* from a packing company, there was a fair sprinkling of *pituitaries;* in this case, we achieved a purification by picking out the pituitaries. This incident relieved our minds about why there was ACTH in these commercial preparations.

With regard to the presence of a factor in pituitary tissue which stimulates aldosterone secretion, I think that there is good reason to believe that such a factor exists. Indeed, we reported, I think it was 2 years ago, that alpha corticotropin, which can be obtained from the pituitary tissue, appears to be a more active stimulator of aldosterone secretion than is beta corticotropin. There is reason to believe, therefore, that the adenohypophysis does contain material which stimulates the secretion of aldosterone.

It is conceivable that in the absence of one gland the other gland may assume greater importance. I think that the pineal cannot be the only source of material which stimulates the secretion of aldosterone since the pinealectomized animal certainly does put out aldosterone; under certain conditions it may put out more aldosterone than does the intact animal. So, the stimulus must come from somewhere else. I think there is a possibility of a general adrenocortical inhibitor arising from the pineal.

Dr. Farber: I would like to ask Dr. Bartter a question and perhaps make a comment about our data, which he so nicely put on a slide for us. How long is the half-life of activity of aldosterone? I ask this question because the data which Eichna and the rest of us collected on these patients with congestive heart failure indicate that the diuresis came very soon after the change in blood pressure which you described; this diuresis was associated, of course, with the pharmacologic action of the digoxin.

Dr. Bartter: I am glad you asked that because I would hate to leave the impression that I had suggested that the only factor controlling sodium retention is aldosterone. As far as the half-life of aldosterone is concerned, if you administer moderate amounts (1 to 16 micrograms) of aldosterone intravenously to an adrenalectomized dog, the effect will be present after an hour and it will be gone in 5 hours.

I am sure that the early sodium diuresi

which you observed is not a result of the inhibition of aldosterone secretion. I am also sure that the sodium retention in cardiac failure is not exclusively, and in some cases perhaps not at all, the result of hypersecretion of aldosterone. Some very interesting results have been reported from Czechoslovakia according to which the use of pharmacologic agents to block the effects of catechol amines sufficed to produce a prompt diuresis in cardiac patients; this observation would suggest that other manifestations of baroceptor activity might influence the renal retention of sodium.

Chairman Sayers: As Dr. Farrell has pointed out, we have found that aldosterone is a very potent stimulant of the myocardium. Its action is immediate. Certainly any improvement in the function of the cardiovascular system will be associated with changes in renal function. I would assume, under these circumstances, that a heightened excretion of sodium may be expected. Therefore, with regard to the administration of aldosterone or fluctuations in the level of this particular steroid, it is not realistic to hold to the narrow view that the hormone is acting only on the kidney; we must expand our horizons, so to speak, and think of aldosterone in terms of an action on other tissues and, in particular, on the myocardium. This might explain some of the discrepancies which have been mentioned.

Dr. Fishman: I should like to ask Dr. Bartter if he is convinced that the systemic arterial *pulse* pressure is actually the regulator of the output of aldosterone. We all know that there are many baroregulatory devices strategically disposed along the vascular tree. These may operate, as in your experiments, to keep *mean* blood pressure constant. Consequently, may not a constant *mean* blood pressure in the face of hemorrhage be a sign of many compensating mechanisms in action? May not a constant mean pressure be masking changes in organ blood flow and volume? May not a change in one of these parameters, which is not reflected in the mean blood pressure, be responsible for heightened aldosterone secretion? Or does the evidence actually pinpoint the pulse pressure as the triggering mechanism?

Dr. Bartter: I am sure that other mechanisms could be involved. But are you referring, in particular, to the blood-loss experiments?

Dr. Fishman: Yes.

Dr. Bartter: Well, that is true of the blood loss, but you can apply constriction of the carotid artery and nothing else whatsoever and get a decrease of carotid pulse pressure but no change in the carotid mean pressure.

Dr. Fishman: If you recorded action potentials from the carotid nerves after artificially decreasing the pulse pressure, I suspect that you would detect an abnormal pattern of discharge. Might not this change in pattern have hemodynamic consequences which would not be reflected in a constant *mean* blood pressure?

Dr. Bartter: I am sure the baroreceptor activity or outflow is there in all its aspects.

Dr. Hilton: What is being alluded to is that you don't know what is going on with relation to the blood flow to the adrenal glands, whether the adrenal glands are ischemic, or if the cardiac output is rising, falling, or what. For example, have you systematically investigated the effects of blood pressure and blood flow on the adrenal secretion of aldosterone?

Dr. Bartter: No. However, systemic arterial blood pressure may either rise (as with carotid constriction) or fall (as with caval constriction) in the face of an increased rate of secretion of aldosterone.

Chairman Sayers: May I interject a point? You have the total secretion, I assume, since you collect a certain volume of adrenal venous blood over a certain time and you measure the concentration of aldosterone in the blood; so, by multiplying concentration by flow, you can calculate the total output of aldosterone over a certain period of time.

Dr. Bartter: Half the output.

Chairman Sayers: Well, then multiply it by 2. I think that answers the question.

Dr. Hilton: Does the adrenal blood flow fall off?

Dr. Bartter: With the constriction of the carotid?

Dr. Hilton: Yes.

Dr. Bartter: No.

Dr. Farrell: I simply want to remark, in connection with Dr. Hilton's question, that in our experience, while it is certainly possible to diminish the secretory rate of the adrenal gland if one cuts off completely the blood supply to the gland, within rather wide limits large variations in adrenal blood flow have no effect on the rate of secretion of aldosterone.

Dr. Mark Nickerson: I just wanted to point out that there is a good deal of old data to indicate that the ordinary baroreceptor reflexes are much more sensitive to any given level of pressure when it is pulsatile than when it is not. I think we can anticipate that, for any given mean pressure, the greater the pulse pressure, the greater the firing of the baroreceptor fibers, and any of the changes that accompany this discharge. The changes may not be primarily in adrenal blood flow, but they certainly affect blood flow through the skin and particularly through the splanchnic area.

On Mathematics in Biology

And since we are assured that the all-wise Creator has observed the most exact proportions, *of number, weight and measure,* in the make of all things; the most likely way therefore, to get any insight into the nature of those parts of the creation, which come within our observation, must in all reason be to number, weight and measure. And we have much encouragement to pursue this method, of searching into the nature of things, from the great success that has attended any attempts of this kind.—Stephen Hales. *Statistical Essays.* Ed. 2, vol. 1, London, W. Innys, 1731, p. 2.

Antidiuretic Hormones

By WILBUR H. SAWYER, M.D., PH.D., ROBERT A. MUNSICK, M.D., AND H. B. VAN DYKE, PH.D., M.D.

There are at least 3 antidiuretic hormones. Arginine vasopressin is the antidiuretic hormone of most mammals, including man. This function is served by lysine vasopressin in the pig, and by arginine vasotocin in birds, reptiles and amphibians. All 3 hormones are closely related chemically and act on the kidney in a similar manner. Arginine vasotocin also exists in fish, in which the antidiuretic response is not known to occur. This antidiuretic hormone appeared, therefore, in vertebrate phylogeny before it acquired antidiuretic function. Anatomic considerations suggest that the fish neurohypophysis serves to regulate adenohypophysial secretion. There is evidence that such a primitive type of neurohypophysial function has persisted throughout subsequent vertebrate evolution.

THE ANTIDIURETIC hormone of man has been chemically identified as arginine vasopressin. This is not, however, the antidiuretic hormone of pigs, birds, reptiles or amphibians. Antidiuresis occurs in these animals in response to neurohypophysial peptides that differ chemically from arginine vasopressin. From a comparative viewpoint, therefore, there is no single antidiuretic hormone but several antidiuretic hormones. All appear closely related chemically and act on the kidney in an analogous manner. An examination of the chemical relationships of these neurohypophysial antidiuretic hormones and their evolution should be of interest and may offer additional insight into their physiologic functions.

Antidiuretic hormones are formed by neurosecretory cells within the hypothalamus and are transported along their axons to be stored in dilated nerve endings closely applied to the capillaries of the neurohypophysis.[1] They appear to be stored in loose combination with protein.[2-4] The chemical form in which antidiuretic hormones are released is not clear. Antidiuretic activity, however, certainly resides in octapeptides such as the vasopressins and their analogues.

The best-defined action of antidiuretic hormones is the promotion of renal tubular reabsorption of free water.[5-9] This action is of major physiologic importance to mammals and probably to nonmammalian terrestrial tetrapods. It is the basic step in the renal mechanism for the conservation of water. Suppression of antidiuretic hormone secretion is, conversely, essential for the production of water diuresis and the elimination of excess water.

Changes in osmotic concentration of blood reaching osmoreceptors within the brain[10] influence the release of antidiuretic hormones in a manner that tends to restore and maintain a stable plasma osmotic pressure. The volume of circulating blood also appears to modify release independently of changes in osmotic pressure in such a manner that water is excreted or retained to restore a normal circulatory volume.[11] Pain, emotion and drugs are among many other factors that may stimulate the release of antidiuretic hormones.

Neurohypophysial Structure and Function in Nonmammalian Vertebrates

Neurohypophysial hormones can cause antidiuresis in mammals, birds, reptiles[12] and amphibians.[13] A similar action in fish, however, has never been demonstrated.[14] The antidiuretic response appears to be an adaptation re-

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Dr. Munsick is a Josiah Macy, Jr. Foundation Fellow, at present in the Department of Obstetrics and Gynecology, Columbia University.

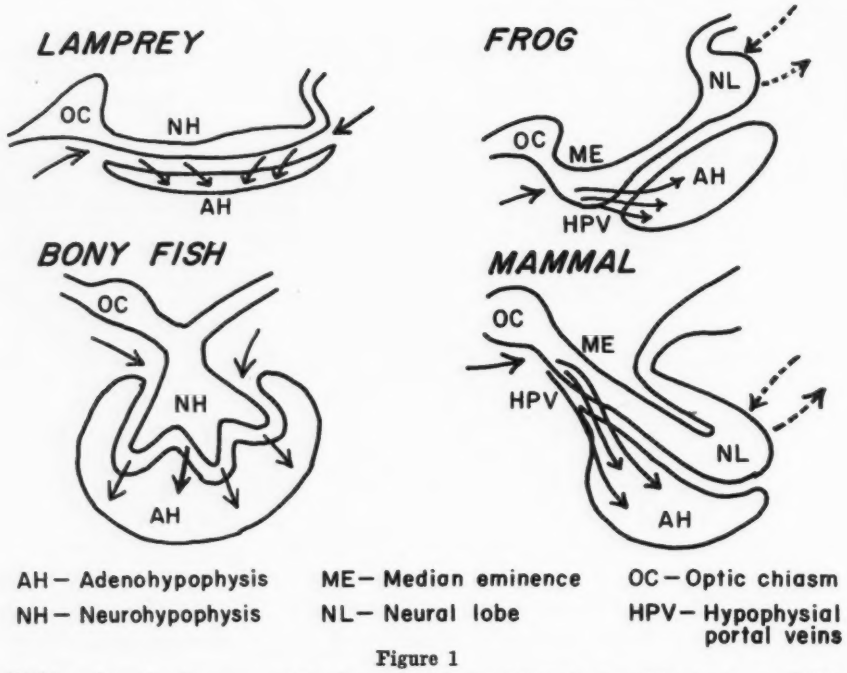

Figure 1

Highly schematic diagrams of the hypophysial circulation in 4 vertebrate types. Solid arrows represent blood flow from neurohypophysis to adenohypophysis. Broken arrows represent the blood flow to and from the neural lobe. (Modified from Green.[16])

lated to terrestrial habitat.[15] The existence of the neurohypophysis and its hormones in fish in the absence of an evident capacity for renal response forces us to search for other functions.

The anatomy of the neurohypophysis in most fish differs strikingly from that in mammals (fig. 1). The neurosecretory nerve endings are either within the tissue of the adenohypophysis or closely applied to the capillaries that lead into the adenohypophysis.[16-19] These neurons seem to be situated so that their secretions must reach the adenohypophysis in high concentrations. The lack of a neural lobe as such in aquatic vertebrates has been interpreted as indicating that the neurohypophysis in such forms functions as an organ of local secretion acting upon the adenohypophysis rather than as an organ for the systemic dissemination of hormones.[16] Neurosecretory endings also occur in lower vertebrates in positions indicating that secretion occurs into the cerebral ventricles and possibly other parts of the brain itself.[1]

The tetrapod neurohypophysis differs from that of fish in the development of a neural lobe (fig. 1). This structure appears, in a primitive form, in certain lungfish[19] and is well developed in anuran amphibians and amniotes. The neural lobe is the caudal end of the neurohypophysis and it is characterized principally by its vascular relationships.[16] Blood traversing its capillary bed does not pass to the adenohypophysis but into the systemic circulation. The neural lobe appears, therefore, well suited to function as an endocrine organ for the storage and general dissemination of neurosecretory hormones.[1]

Local Actions of Neurohypophysial Hormones

The rostral portion of the neurohypophysis retains, even in mammals, an intimate relationship to the vascular supply of the adenohypophysis. Most, if not all, of the blood

reaching the adenohypophysis passes first through the capillaries of this portion of the neurohypophysis, the median eminence, before being carried to the adenohypophysis by portal vessels. This certainly suggests that neurohypophysial secretions retain an action upon the adenohypophysis, and the physiologic evidence supporting this is growing rapidly.

Injection of huge doses of vasopressin intravenously causes increased adrenocortical secretion, possibly due to stimulation of ACTH release from the pituitary gland[20] and from storage in other tissues,[21] and to a direct action upon the adrenal glands.[22] Relatively small doses of arginine vasopressin instilled into the cavity of the third cerebral ventricle, however, appear to cause elevation of the rate of ACTH secretion in conscious dogs.[23] Equimolar doses of 3 closely related peptides, oxytocin, arginine vasotocin (3-isoleucine, 8-arginine vasopressin) and oxypressin (8-leucine vasopressin), do not cause ACTH release when injected into the third ventricle. It is reasonable to believe that arginine vasopressin can diffuse from the third ventricle into the hypophysial portal system and is carried to the adenohypophysis in relatively high concentration, causing ACTH release. Less direct action by way of other cerebral structures cannot, however, be excluded.

That arginine vasopressin in low doses can cause ACTH release when applied close to the adenohypophysis is far from proof that it is the neuroendocrine agent in hypophysial portal blood that causes ACTH secretion.[24] Guillemin and others[25] have obtained preparations from neurohypophysial extracts in which vasopressor activity is considerably reduced relative to ACTH-releasing ability. It appears quite possible that releasing factors exist that are closely related to vasopressin or are derivatives of vasopressin. Whether they are physiologic mediators of secretory stimuli from the hypothalamus to the adenohypophysis remains to be demonstrated. This suggests, however, that neurosecretion of vasopressin-like peptides into the portal circulation may be an important physiologic link in the control of one adenohypophysial function by higher nerve centers.

A growing body of evidence suggests that oxytocin may influence adenohypophysial secretion of gonadotropins. Systemic oxytocin in large doses delays mammary involution in nursing animals after removal of the litter, presumably by maintaining luteotropin (prolactin) secretion.[26, 27] Oxytocin can also induce pseudopregnancy in rats,[28, 29] indicating that it can initiate pituitary luteotropin secretion. Large intravenous doses of oxytocin or vasopressin are reported to increase gonadotropin excretion in rabbits.[30] It is conceivable, therefore, that oxytocin or oxytocin-like neurosecretions provide a means of hypothalamic control over gonadotropin secretion by the adenohypophysis analogous to the possible action of vasopressin on the adenohypophysial secretion of ACTH.

The suggestion that vasopressin suppresses thirst[31] is an interesting one, although the evidence for this action is not strong. This would represent an action on higher nervous centers, modifying subjective sensations and behavioral responses. Vasopressin and oxytocin administered systemically in large doses are capable of causing spawning behavior in some fish, even after hypophysectomy and castration.[32] This is clearly an action on the central nervous system although it may not be a direct one. That oxytocin may retain actions directly upon cerebral structures in mammals is suggested by the extraordinary observation of Brooks and Pickford[33] that small doses of oxytocin injected into the carotid circulation of conscious dogs produce a marked and persistent elevation of renal sodium excretion. Similar doses administered systemically are without appreciable effect.

Nature of Neurohypophysial Hormones in Mammals

The architecture of neurohypophysial tissue of mammals differs from that of other vertebrates. It is also of interest to inquire into pharmacologic differences and similarities with the expectation that information obtained

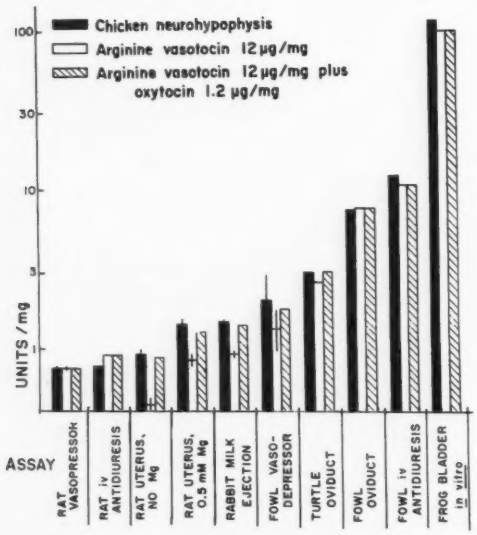

Figure 2

The activities found in chicken neurohypophysial extract on several assays. These activities are also shown for arginine vasotocin alone and for arginine vasotocin combined with oxytocin in the proportions in which they are believed to exist in the chicken extract. Units, in each assay, are based on comparison with U.S.P. Posterior Pituitary Reference Standard on that assay.

may increase our understanding of neurohypophysial function in mammals.

Arginine vasopressin has been chemically identified in the neurohypophyses of man,[34] cow,[35] sheep,[36] and horse.[37] Although there is no chemical proof, there is sound pharmacologic evidence for the presence of arginine vasopressin in monkey, cat, dog, rabbit, rat, and camel,[38] and preliminary pharmacologic evidence for its presence in the opossum.[39] The single known exception to this rule among the mammals is the domestic pig.[40] The neurohypophysis of this animal contains lysine vasopressin. Since hog pituitaries are an important commercial source of vasopressin solution, one rarely knows, when he cracks an ampule, whether he is about to administer arginine or lysine vasopressin or a mixture of the 2. Whether this distinction is only academic as far as human patients are concerned has not been established.

As the antidiuretic hormone secreted by the pig, lysine vasopressin appears to be at least as potent as arginine vasopressin in causing antidiuresis in this species.[41] Arginine vasopressin is, however, many times more potent than lysine vasopressin in causing antidiuresis in the dog.[42] The rat is less discriminating, but the antidiuresis which results from lysine vasopressin is considerably briefer than that which occurs after arginine vasopressin.[43–45] The antidiuretic responses to the 2 vasopressins in man have not been compared.

Neurohypophysial Hormones in Nonmammalian Vertebrates

Neither lysine nor arginine vasopressin appears to be present in those nonmammalian vertebrates that have been adequately studied.[46] Pharmacologic and chromatographic studies on the chicken neurohypophysis have led to the conclusion that there are at least 2 active principles.[47–48] One appears to be oxytocin. The second has pharmacologic activities that clearly distinguish it from any known mammalian hormone (fig. 2). We were extremely fortunate to have received, from Dr. du Vigneaud's group at Cornell Medical College, many structural analogues of neurohypophysial hormones for pharmacologic study.[49] Among these analogues was one called "arginine vasotocin." This chemical hybrid was synthesized by Katsoyannis and du Vigneaud.[50] It has the ring structure of oxytocin and the side-chain of arginine vasopressin. The pharmacologic properties of arginine vasotocin match quite precisely the properties of the second principle in chicken neurohypophysial extract. If this pharmacologic evidence can be substantiated chemically through the isolation and identification of arginine vasotocin in the chicken neurohypophysis, Drs. Katsoyannis and du Vigneaud are in the extraordinary position of having synthesized a polypeptide hormone without being aware of its existence in nature.[51]

Arginine vasotocin appears to be present in a greater quantity than oxytocin in the chicken neurohypophysis.[47] It is much more potent than oxytocin in causing antidiuresis,

uterine contraction, and oviposition in the hen. This would indicate that arginine vasotocin is the antidiuretic hormone of the chicken and, possibly, is also involved in the process of egg-laying. The function of oxytocin in the hen remains obscure.

Employing similar methods with neurohypophysial extracts from a reptile, we have also tentatively identified arginine vasotocin and oxytocin.[46] We have also obtained pharmacologic evidence that neurohypophyses from 2 anuran amphibians, a frog and a toad, contain arginine vasotocin and a smaller quantity of oxytocin.[46, 52]

Bony fish appear to have active principles in their neurohypophyses quite similar, if not identical, to the principles in amphibians.[46, 48, 53] Although arginine vasotocin is present, its function in fish remains a mystery. No clear-cut influence on renal function or water metabolism has been described.[14, 54] Depletion of neurosecretory material in hypothalamic nuclei and neurohypophyses in fish exposed to extremely concentrated external environments affords but weak evidence for a specific role of such secretion in the defense of internal osmotic integrity.[55, 56]

The elasmobranch neurohypophysis contains a peptide with biologic activities quite distinct from those of any of the neurohypophysial hormones or analogues that we have studied.[46, 57] There is no evidence that would suggest a function for neurohypophysial secretion in elasmobranchs.

The cyclostomes represent living remnants of the jawless fish that were ancestors of all vertebrates. The neurohypophysis of the marine lamprey, a member of this group, contains a spectrum of pharmacologic activities indistinguishable from that of arginine vasotocin.[46, 58] There is little or no oxytocin present.[46] Again we have no information concerning the function of this peptide in the lamprey.

The Search for Related Principles in Invertebrates

Since active polypeptide principles occur in all major vertebrate groups, it is of interest to look for related substances among the invertebrates. Tunicates are believed to be closely related to the ancestors of the vertebrates. The large solitary ascidians (fig. 3) contain a structure that has a certain superficial resemblance to the vertebrate pituitary. This is the neural complex, made up of the remains of the larval brain, called the cerebral ganglion, and a closely applied glandular structure, the subneural gland. This gland has a duct which communicates with the pharynx. The analogy of this association to that of the neural and glandular portions of the vertebrate hypophysis has tempted many to suggest homology of function.[59, 60] The presence of neurosecretory cells within the cerebral ganglion also lends support to this hypothesis.[61] Neurohypophysial-like activities have been sought and found in extracts of the neural complex.[62, 63] More critical investigations, however, reveal that the same weak oxytocic activity can be found in any tissue of the ascidian[64-66] and that milk ejection, pressor and antidiuretic activities are essentially nonexistent. The oxytocic activity resists alkali and sodium thioglycolate, unlike any known neurohypophysial peptide. The pharmacologic similarities between the neural complex and the neurohypophysis appear too superficial to offer any support for the argument in favor of homology.[65, 66]

We have, therefore, no knowledge of the prevertebrate evolution of the neurohypophysis. Neurosecretory systems analogous to the hypothalamic-neurohypophysial system are widespread among invertebrates.[1] There is fragmentary evidence that certain crustaceans have neurosecretory products that can cause water uptake by frogs.[67, 68] This might indicate that principles similar to neurohypophysial hormones may exist in invertebrates.[1] It seems extraordinary that such could be found in the eyestalks of crustaceans which are believed to be very distantly removed from the line of evolution leading to the vertebrates. We are left, therefore, with the thought that neurosecretory hormones may, throughout evolution, have common chemical characteristics.[1] The alleged hormone in crustaceans that has frog water-balance activity may be an

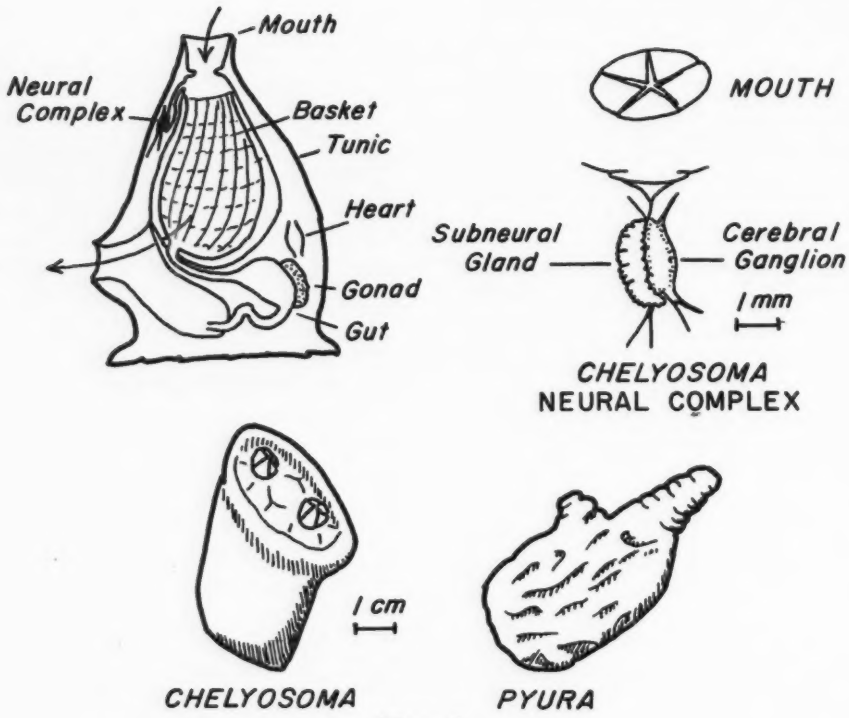

Figure 3

Top left. *Diagrammatic representation of a typical solitary ascidian.* Top right. *The neural complex of Chelyosoma.* Bottom. *Sketches of 2 large ascidians that have been used for the pharmacologic investigation of the presence of activities resembling those of the neurohypophysial hormones.*

example of convergent evolution of neurosecretory hormones. Neurosecretory products resembling vertebrate neurohypophysial hormones may have existed in the invertebrate ancestors of the vertebrates. However, their presence in surviving forms related to the early vertebrates remains to be demonstrated.

Vertebrate Evolution and Neurohypophysial Peptides

Active neurohypophysial peptide hormones are present in all the major vertebrate groups. The active principles differ, however, from group to group. They have been chemically identified only in a few mammals. We have studied a small number of nonmammalian species pharmacologically. Using this pharmacologic evidence, it might be possible to formulate a tentative scheme for the evolution of neurohypophysial peptides based on the assumption that if 2 species share a peptide their most recent common ancestor also possessed that peptide. On this basis a phylogenetic scheme for neurohypophysial peptides is set forth in figure 4.

The *Agnatha* or cyclostomes are the most primitive living vertebrates. These jawless fish have a very primitive neurohypophysis that appears to contain arginine vasotocin. Proximity of the neurohypophysis to the vascular supply of the adenohypophysis suggests that neurohypophysial secretion in this primitive form is carried directly to the adenohypophysis. It is plausible to postulate, therefore, that the vertebrate neurohypophysis first appeared as an organ for local secretion modulating adenohypophysial functions.

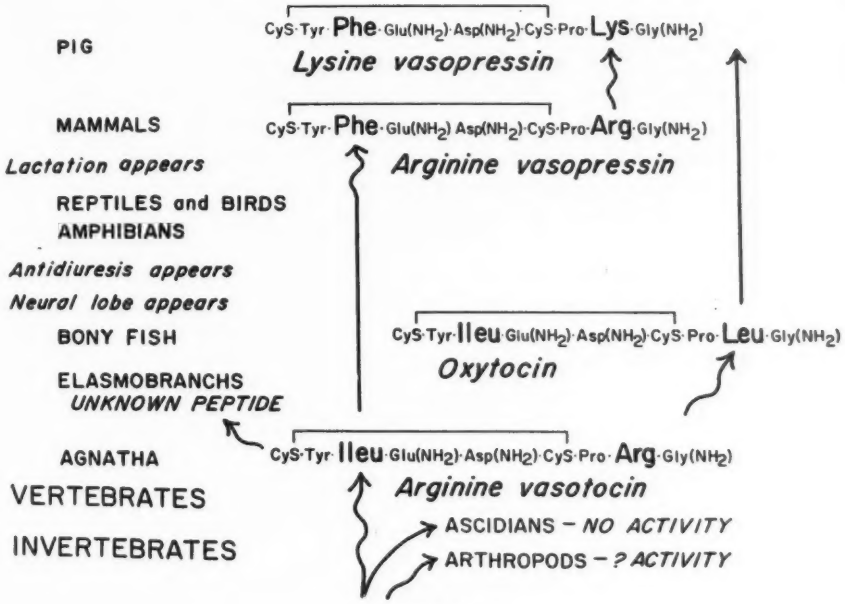

Figure 4

Hypothetic diagram of the phylogenetic relationships among neurohypophysial peptides.

Sharks and rays represent a line of evolution of jawed fish divergent from that leading to the bony vertebrates. In this group, a neurohypophysial peptide is present that is pharmacologically unique. We have no information concerning its chemical nature or its physiologic function.

The pharmacologic evidence indicates that the bony fish have retained arginine vasotocin. In addition, oxytocin occurs in this group, perhaps having evolved from arginine vasotocin by the substitution of leucine for arginine in the penultimate position of the side-chain. We have but meager clues as to the physiologic functions of these peptides in bony fish. The architecture of their pituitaries suggests that they function as local mediators between the neural and glandular divisions of the hypophysis rather than as systemic hormones.

The amphibians appear to have inherited the same peptides that are present in bony fish. They have, however, added the neural lobe, apparently as a structure for the systemic dissemination of neurosecretory products. Here one now finds that arginine vasotocin has acquired striking effects on the water permeability of the skin, the bladder, and the distal renal tubule. These actions promote water uptake through the skin, water reabsorption from the bladder, and water conservation by the kidney. Antidiuresis appears first, therefore, in amphibians as 1 of 3 mechanisms available for the retention and absorption of water and the protection of osmotic integrity. Arginine vasotocin appears to be the antidiuretic hormone of amphibians and presumably contributes to their ability to be amphibious.

Reptiles and birds do not seem to have altered the neurohypophysial peptides inherited from the amphibians. They have retained the antidiuretic response to arginine vasotocin. This peptide is also active in causing contraction of the oviduct in reptiles and birds. The function of oxytocin in these forms remains obscure.

Mammals have abandoned arginine vasotocin. Arginine vasopressin has displaced it as

the antidiuretic hormone. This step represents but a single amino acid substitution in the third position of the ring where phenylalanine replaces isoleucine. The domestic pig is the single known exception. Here lysine replaces arginine as the penultimate amino acid of the side-chain. This seems to be an isolated mutation, perhaps similar to that which occurred in the elasmobranchs.

Following its appearance in the bony fish, oxytocin persisted throughout subsequent evolution, its structure apparently inviolate. Its only established physiologic functions are related to lactation and, possibly, to mammalian parturition. Lactation is, of course, exclusively mammalian. Oxytocin does not appear to be directly concerned with egg-laying in birds or other nonmammalian vertebrates. It appears, therefore, that oxytocin evolved in the fish millions of years before the oxytocic or milk-ejection responses.

F. L. Hisaw[69] recently restated his generalization that, "It is not hormones which have evolved but the uses to which they are put." Although the primitive uses of oxytocin in nonmammalian vertebrates are unknown, new uses certainly evolved in the mammals. Arginine vasotocin is also present in fish, but antidiuresis first appeared in amphibians or their lungfish ancestors. This is another example of an ancient molecule adapted to a new use.

Hisaw also emphasized that his rule, like any good rule, does have exceptions. Antidiuretic hormones appear to offer an example of hormones that did undergo evolution while the "uses to which they are put" did not. The identity of the antidiuretic hormone changed twice during evolution, once from arginine vasotocin to arginine vasopressin and, in the pig, again to lysine vasopressin. The mechanism of antidiuretic action and its physiologic function, however, remained unchanged.

Neurohypophysial peptides evolved, therefore, long before their known peripheral actions. The primitive neurohypophysis appears to be an organ for local secretion into the blood supply of the adenohypophysis. This observation suggests that the neurohypophysis originated as a neurosecretory link between the brain and the adenohypophysis. A portion of the mammalian neurohypophysis retains a primitive anatomic relationship to the adenohypophysial circulation. We must seriously entertain the hypothesis that the mammalian neurohypophysis also retains a primitive physiologic function as a source of chemical mediators of hypothalamic regulation of adenohypophysial activities.

The term "antidiuretic hormone" is an imprecise one, necessitating a parenthetic identification. If reference is made to arginine (or lysine) vasopressin, the amino acid should be included in the name in order to define the hormone's chemical identity. Antidiuretic hormones appeared, however, far earlier in vertebrate phylogeny than did the antidiuretic response, and not as vasopressin. We would be wise, therefore, if we continue to use the term "antidiuretic hormone" or "ADH," to recognize that in the use of the adjective "antidiuretic" we may be prejudicing ourselves by emphasizing a recently acquired use to which these hormones have been put and discouraging our curiosity about other more primitive functions of possible physiologic importance.

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Down the Drain

Elongated particles like threads or rods have a great tendency to associate and form structures. Such association is favored by the shape, the large surface, the poor balance of forces, and the friction resulting therefrom. Low motility and high viscosity will be characteristic of these particles and nature will invariably resort to this form wherever it wants to build structures. The "structure" of the cell is built of such particles and the really basic biological functions are linked to structure. Particles within this structure are mostly so intimately interlaced that they cannot be disentangled and isolated without profound damage. If we extract cells or tissues with water, a considerable part, the smaller half of the protein will be dissolved, but it will be globular proteins only which pass into solution while the basic structure will be left behind in the form of a semi-solid mass. Researchers did not know what to do with it and resolved the difficulty mostly by calling it "residue" and sending it down the sink. Unconsciously, research limited its attention therefore to the easily accessible globular proteins performing rather secondary functions around the basic structure and what we call today "protein chemistry" is, in its greatest part, only the chemistry of globular proteins.—A. Szent-Gyorgyi. *Nature of Life. A Study on Muscle.* New York, Academic Press, 1948, p. 10.

Adrenocorticotropic Action of Antidiuretic Hormone

By JAMES G. HILTON, M.D.

By means of direct arterial perfusion of the adrenal glands of the hypophysectomized dog, it has been shown that synthetic lysine, arginine and acetyl arginine vasopressins stimulate the adrenal cortex directly to secrete hydrocortisone. Pressor activity and cortisol-stimulating activity were demonstrated to be independent of each other. Similar polypeptides, such as oxytocin, insulin, glucagon, and pressor amines, such as epinephrine and norepinephrine, did not show any cortisol-stimulating activity. ACTH was found consistently to increase the rate of secretion of aldosterone when perfused through the glands of hypophysectomized animals. The probable role of arginine vasopressin as an important factor in the stress reaction is considered along with its postulated ability to activate adrenal phosphorylase.

WE HAVE previously reported our preliminary experiments which show that synthetic vasopressin, when injected into the arterial circuit of the adrenal glands of the hypophysectomized dog, stimulates directly adrenal cortical secretion.[1, 2] In this paper I should like to report the results of this work together with additional experiments designed to elucidate the mechanism of the adrenocorticotropic action of vasopressin. Associated with me in this work have been Dr. Louis F. Scian, who is a Fellow of this Association, Dr. Coenraad Westermann and Dr. Oscar Kruesi.

Methods

Adult mongrel dogs were anesthetized by the intravenous administration of 0.5 mg. per Kg. body weight of sodium pentobarbital. When desired, hypophysectomy was done by the transbuccal approach of McClean.[3]

In all experiments, the adrenal glands were perfused using the *in situ* technic of Hilton et al.[4] In this preparation, arterial heparinized blood obtained from donor dogs is pumped at a constant rate of 10 ml. per minute into the arterial circuit of the isolated adrenal glands of the recipient animal (fig. 1*A*). At the start of perfusion by the pump, the heart of the recipient animal is fibrillated so that the glands are perfused solely by donor blood for the duration of the experiment. Side arms on the tubing leading into the arterial circuit of the adrenal glands allow for the continuous recording of blood pressure within the isolated adrenal pouch preparation, as well as for the injection or infusion of various agents.[4]

The venous outflow from the adrenal veins was collected in iced graduated cylinders. In none of the experiments was any of the venous effluent from the adrenal glands allowed to recirculate or to return to the general circulation of the recipient (fig. 1*B*). The rate of secretion of hydrocortisone was calculated as the product of the rate of venous blood flow and the arteriovenous difference in the concentration of hydrocortisone.

After several control collections, the agents under study for their adrenocorticotropic activity were infused via a side arm in the arterial perfusing circuit and additional collections were made.

At the end of each experiment, an injection of ACTH was given to verify adrenal viability and to compare the rates of secretion of hydrocortisone with those of the previous infusion periods.

The concentrations of hydrocortisone were determined by the method of Peterson et al.[5] The concentrations of aldosterone in adrenal venous blood were measured by a double isotope dilution technic.*

Results

Experiments with Synthetic Lysine Vasopressin

Six experiments were done. They are summarized in table 1. Because of the variability in responsiveness of the adrenal glands to the standard dose of ACTH from 1 dog to the

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*We are indebted to Dr. James O. Davis of the National Heart Institute for these determinations.

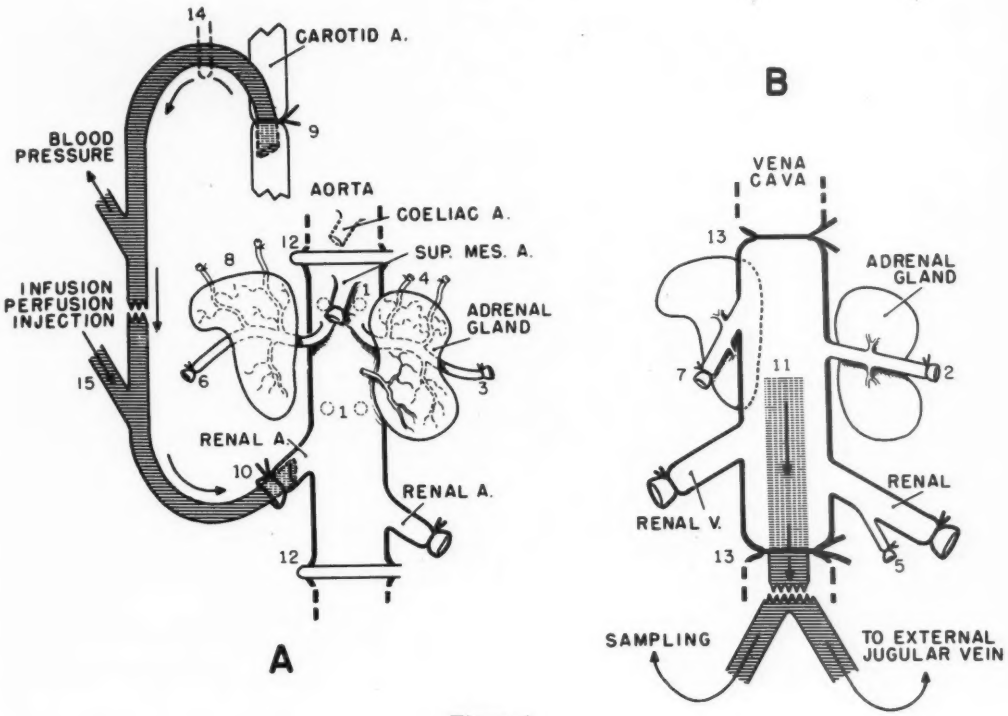

Figure 1

Diagram of adrenal arterial and venous perfusion pouches. In A *the circuit is clamped off at* 14 *and blood from a donor animal is pumped into the aortic pouch via side arm* 15. *In* B *venous blood is* not *returned to the external jugular vein. (Republished by permission of the American Journal of Physiology.*[4]*)*

Table 1

Comparison of the Increment in the Rate of Secretion of Hydrocortisone (ΔF) *Following Lysine Vasopressin with that Following ACTH*

Experiment no.	Before ACTH F μg./min.	Lysine vasopressin dose	duration	ΔF μg./min.	After ACTH* ΔF μg./min.	Vasopressin† responsiveness
		HYPOPHYSECTOMIZED				
1	0.3	0.22 U./min.	10 min.	0.7	2.0	35%
2	0.3	0.3 U./min.	7 min.	1.6	3.5	46%
3	0.2	0.3 U./min.	7 min.	3.3	11.0	30%
		NONHYPOPHYSECTOMIZED				
4	2.6	0.22 U./min.	10 min.	6.2	8.1	77%
5	9.2	0.16 U./min.	7 min.	4.0	2.3	174%
6	6.0	0.2 U./min.	7 min.	2.4	1.9	126%

*One unit per minute for 7 minutes.

†$\frac{\Delta F \text{ after vasopressin}}{\Delta F \text{ after ACTH}} \times 100.$

next, we have tabulated the results in terms of the increment (ΔF) in the secretion of cortisol which occurred after the administration of ACTH. This increment in the secretion of cortisol above the control rate of secretion gives a measure of the maximal responsiveness or sensitivity of the glands in each dog to the supramaximal dose of ACTH, i.e., 1 unit

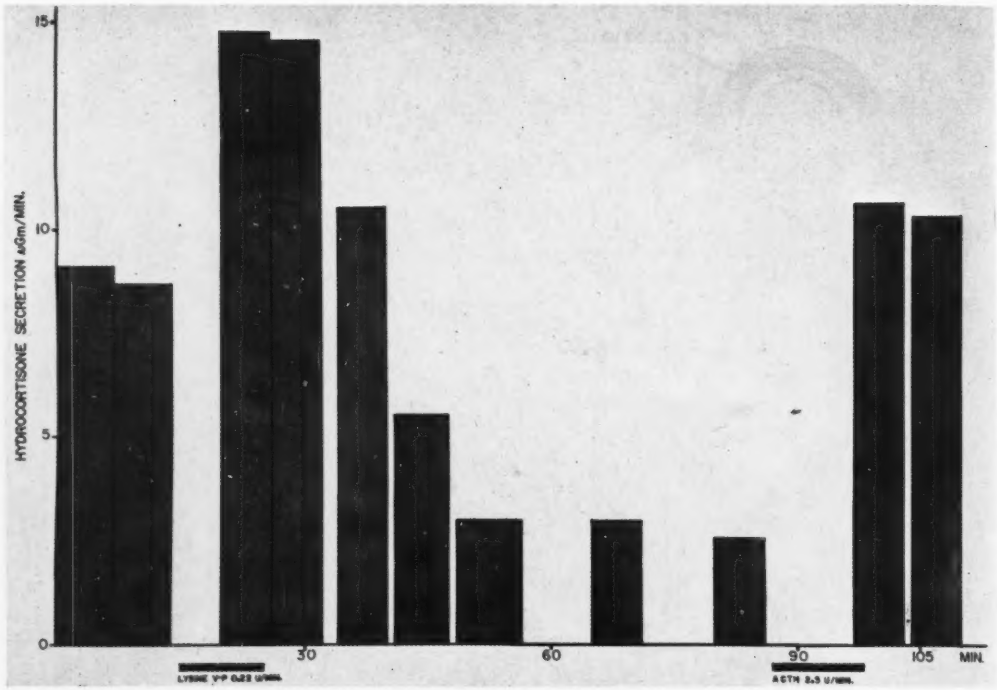

Figure 2

Experiment showing effect of lysine vasopressin on the rate of secretion of hydrocortisone. Adrenal glands of a nonhypophysectomized dog are perfused with blood from a hypophysectomized donor.

Table 2

Effects of Graded Doses of Arginine Vasopressin on the Increment in the Rate of Secretion of Hydrocortisone (ΔF) in Hypophysectomized Dogs

No. experiments performed	Before ACTH av. F µg./min.	Arginine vasopressin dose (U./min.)*	Arginine vasopressin av. ΔF µg./min.	After ACTH† av. ΔF µg./min.	Vasopressin‡ responsiveness
2	<0.1	0.001	0.4	3.1	13%
2	<0.1	0.01	1.6	4.1	39%
3	<0.1	0.1	2.5	3.6	69%
2	0.15	0.4	4.5	3.8	118%

*All doses given over a 7-minute period.
†One unit per minute for 7 minutes.
‡$\frac{\text{av. } \Delta F \text{ after vasopressin}}{\text{av. } \Delta F \text{ after ACTH}} \times 100.$

per minute for 7 minutes.* The increment in the secretion of cortisol following the administration of vasopressin was then compared with that following the administration of ACTH and expressed as a percentage of the ACTH response. As may be seen in table 1, the amount of vasopressin perfused into the arterial circuit of the glands varied from 0.16 U./min. to 0.3 U./min. for 7 to 10 min-

*In our preparation, the secretion of hydrocortisone first becomes detectable following 1.0 mU. of ACTH and becomes maximal at 10 mU. All injections in this type of assay were given as single doses of 1 ml. each. There was no recirculation of the ACTH through the glands.[11]

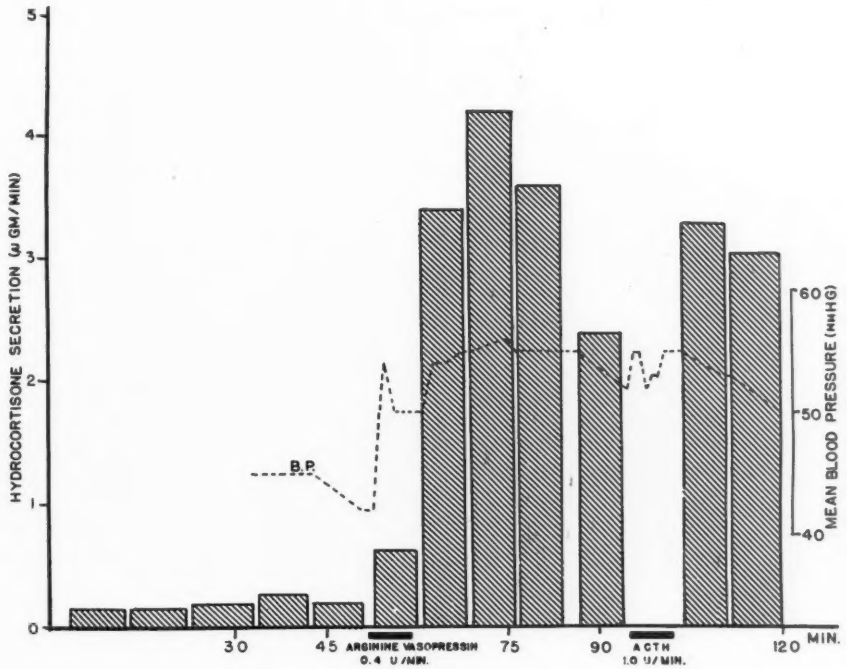

Figure 3

Effect of arginine vasopressin. Adrenal glands of a hypophysectomized dog are perfused with blood from a hypophysectomized donor.

utes. There did not appear to be any correlation between these small differences in dose and the magnitude of stimulatory effect on the secretion of cortisol.

Figure 2 depicts the complete protocol of 1 of these experiments (no. 4, table 1). In this experiment, a nonhypophysectomized recipient animal was perfused with blood from a hypophysectomized donor. In the 2 control periods, before the administration of vasopressin, the rate of secretion of cortisol averaged 8.8 μg./min. After vasopressin, there was an increment in the rate of secretion to a level of 14.8 μg./min.

In this series of synthetic lysine vasopressin experiments, the mean blood pressure in the adrenal arterial circuit rose during the administration of the hormone by an average of 27 mm. Hg, with a range of 8 to 41 mm. Hg. There was no pressor activity associated with the administration of ACTH.

Experiments with Synthetic Arginine Vasopressin

Figure 3 depicts a typical example of this group of experiments. Both recipient and donor animals had been completely hypophysectomized; consequently, control cortisol secretion rates were very low, averaging less than 0.2 μg./min. Following the injection of arginine vasopressin, the rate of secretion of cortisol rose to a peak of 4.2 μg./min. representing an increment of 4.0 μg./min. above control. Mean blood pressure rose by an average of 10 mm. Hg.

In an attempt to define a dosage-response relationship, 8 additional experiments were performed in completely hypophysectomized animals. These experiments are summarized in table 2, which is similar in design to table 1. There is a good correlation between the dose of vasopressin and the response of the glands.

Since in all experiments thus far the vasopressin had been injected only over a 7-minute

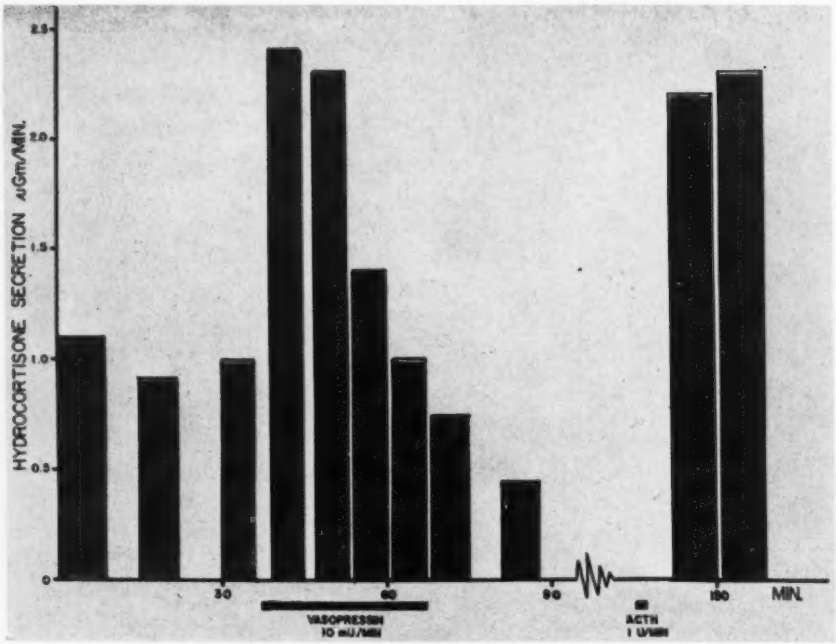

Figure 4

Effect of 30 minutes of perfusion of arginine vasopressin on the rate of secretion of hydrocortisone in the hypophysectomized dog.

Table 3

Rate of Secretion of Hydrocortisone (F) *and Aldosterone µg./min. Following Injections of Various Synthetic Vasopressins**

Experiment	Vasopressin dosage over 7-minute period	Control F	Control aldosterone	After vasopressin F	After vasopressin aldosterone	After ACTH F	After ACTH aldosterone
1	lysine 0.3 U./min.	0.35	0	1.9	0	3.8	0.015
2	lysine 0.3 U./min.	0.6	0.005	3.5	0.008	11.2	0.029
3	acetyl arginine 14.3 µg./min.	0.6	0.01	6.5	0.045	6.0	0.057
4†	acetyl arginine 28.6 µg./min.	4.3	0.163	11.0	0.141	10.2	0.132

*ACTH was given at the end of each experiment at a rate of 1 U./min. for 7 minutes. All animals were hypophysectomized except as noted.

†Donor animal incompletely hypophysectomized at time of bleeding.

period, and since the stimulatory effect of vasopressin had more or less disappeared within 20 minutes after the end of the injection, the effect of a prolonged infusion of arginine vasopressin on the rate of secretion of cortisol was determined. Figure 4 illustrates the results which were obtained when such an experiment was performed on a hypophysectomized animal. Despite continuation of the vasopressin infusion (10 mU./min.) for 30 minutes, the rate of secretion of cortisol began to fall to control levels *during* the infusion.

Experiments Concerned with the Rate of Secretion of Aldosterone

Four experiments were performed and are summarized in table 3. It should be noted that in 3 of the experiments there was a definite increase in the rate of secretion of aldosterone following ACTH. In experiment 4 the donor animal was found to have a small remnant

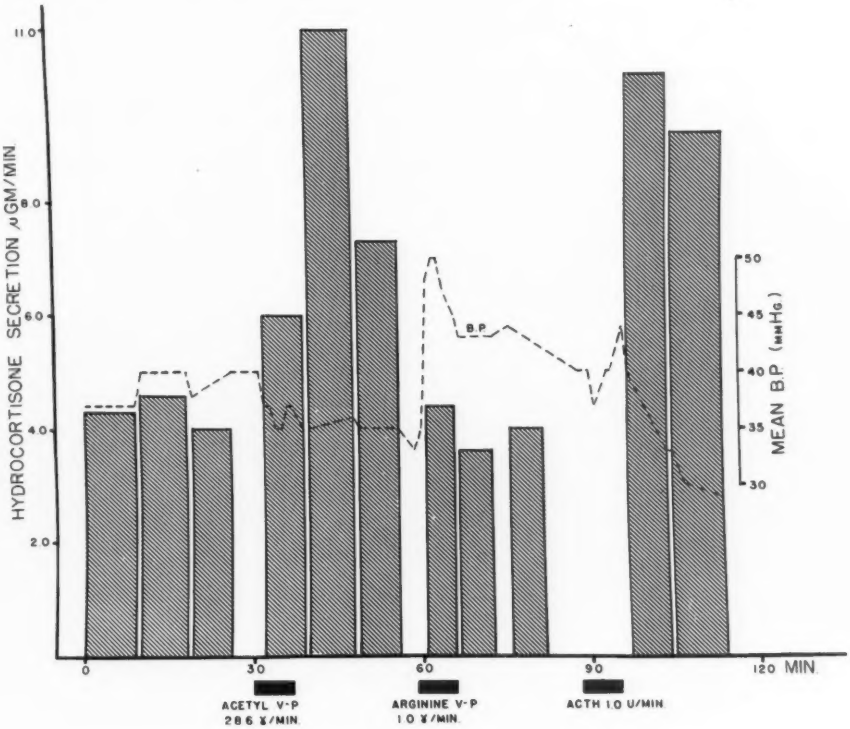

Figure 5

Comparison of the effects of acetyl arginine vasopressin (28.6 µg./min. for 7 minutes) and of arginine vasopressin (1.0 µg./min. for 7 minutes) on the mean blood pressure in a hypophysectomized dog.

of pituitary tissue after hypophysectomy. Apparently this remnant was insufficient to produce a maximal secretion of cortisol in the perfused adrenal glands, since there was a further rise after the administration of vasopressin and ACTH, but was sufficient to maintain a sustained, high rate of aldosterone secretion.

Although lysine vasopressin (experiments 1 and 2) showed its usual stimulatory effect on the secretion of cortisol, it was without effect on the secretion of aldosterone. On the other hand, acetyl arginine vasopressin showed both hydrocortisone- and aldosterone-stimulating activity as may be seen in experiment 3, table 3. However, acetyl arginine vasopressin has no pressor activity, a phenomenon illustrated in figure 5. When arginine vasopressin was subsequently injected, the expected rise in mean blood pressure occurred without a rise in the secretion of cortisol. It is possible that the acetyl analogue blocked, in some manner, the usual stimulatory effect of arginine vasopressin on the secretion of hydrocortisone by the perfused adrenal glands without blocking its pressor activity on the blood vessels of the adrenal glands. Further investigations of this question of blocking action, as well as of the effects of arginine vasopressin itself on aldosterone secretion, are needed for definitive conclusions.

Experiments in Which Miscellaneous Compounds Were Studied

It was considered of interest to see whether other polypeptides, similar or dissimilar to vasopressin, might have cortisol-stimulating

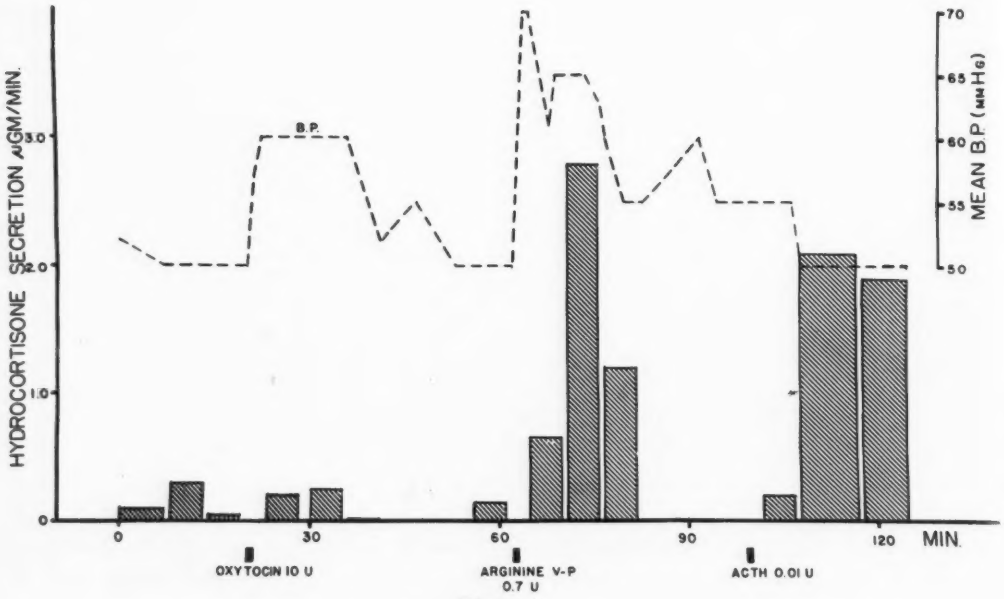

Figure 6

Comparison of the effects of synthetic oxytocin, arginine vasopressin and ACTH on the secretion of hydrocortisone and the mean blood pressure in the hypophysectomized dog.

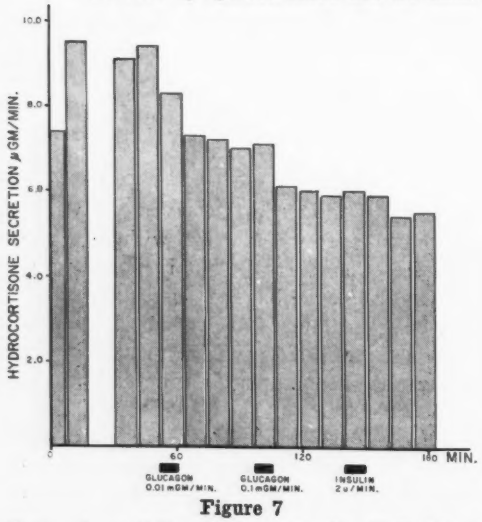

Figure 7

Comparison of the effects of insulin (2 U./min.) and glucagon (0.01 mg./min. and 0.1 mg./min.) on the rate of secretion of hydrocortisone in the nonhypophysectomized dog.

activity. In 4 experiments (2 with hypophysectomized and 2 with nonhypophysectomized preparations), synthetic oxytocin, in a dosage ranging from 10 U./min. for 1 minute to 1.4 U./min. for 7 minutes, had no activity. At these high doses there was, however, pressor activity as seen in the experiment depicted in figure 6.

In 2 experiments with hypophysectomized and in 3 experiments with nonhypophysectomized animals, insulin, in a dosage of 1 U./min. to 80 U./min for 1 to 10 minutes, had no effect on either the mean blood pressure or the secretion of cortisol (fig. 7).

Glucagon, in 6 hypophysectomized and 14 nonhypophysectomized preparations, had no effect either on the rate of secretion of cortisol or on the mean blood pressure (fig. 7). Dosages used ranged from 0.01 mg./min. to 1.0 mg./min. for 1 to 38 minutes.

Finally, norepinephrine (4 µg./min. for 7 minutes) and epinephrine (2 µg./min. for 7 minutes) had no effect on the rate of secretion of cortisol. The former compound did, however, cause a rise in mean blood pressure of 15 mm. Hg in the perfused adrenal gland (fig. 8).

3′,5′-adenosinemonophosphate (3′,5′-AMP) has been shown to have potent adrenocortico

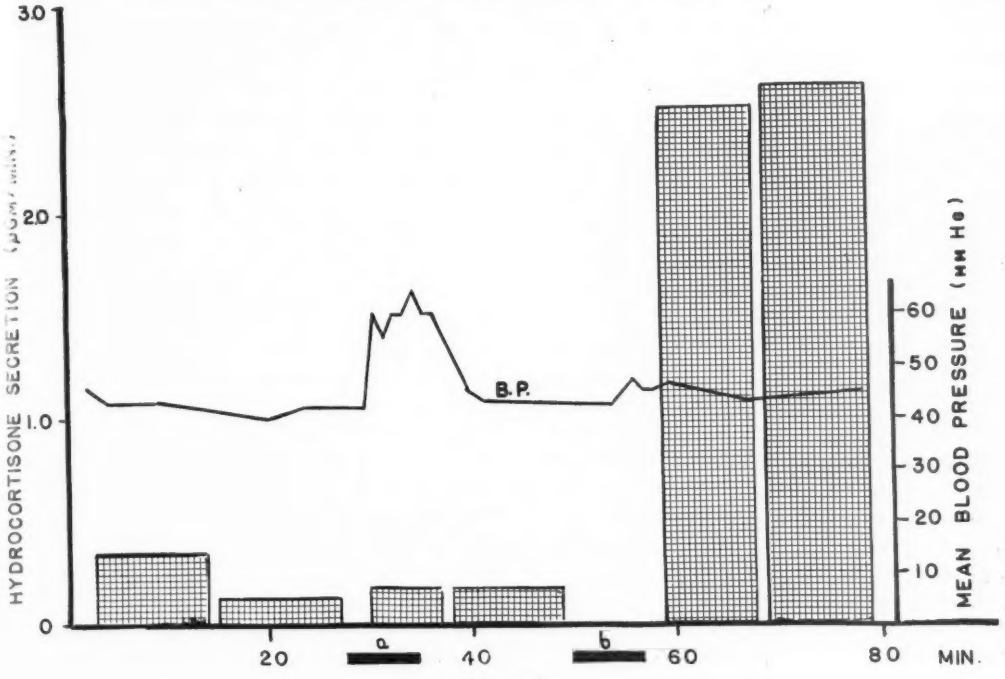

Figure 8

Comparison of the effects of norepinephrine and ACTH on the rate of secretion of hydrocortisone. At a *norepinephrine was injected at a rate of 0.1 μg./min. At* b *1.0 U./min. of ACTH was administered. Animal hypophysectomized.*

tropic activity.[8, 9] Arginine vasopressin was injected into an adrenal gland preparation which was being perfused with 3′,5′-AMP at 0.1 mg./min. The cortisol-stimulatory activity of 3′,5′-AMP is illustrated in figure 9. Following each injection of arginine vasopressin (100 mU./min. for 7 minutes), there was a transient increment in the rate of secretion of cortisol of 2.1 μg./min. and 1.1 μg./min. respectively. Immediately following cessation of the 3′,5′-AMP infusion, cortisol secretion fell to basal levels.

It is known from the experiments of Haynes[8] that ACTH causes the accumulation of 3′,5′-adenosinemonophosphate in incubates of beef adrenal cortex and that this, in turn, stimulates the production of adrenal phosphorylase. Haynes, Koritz and Péron[9] have also shown that 3′,5′-AMP added to the adrenal glands of the rat which have been incubated in vitro elicits an increase in the rate of production of corticosteroid which is equal to,

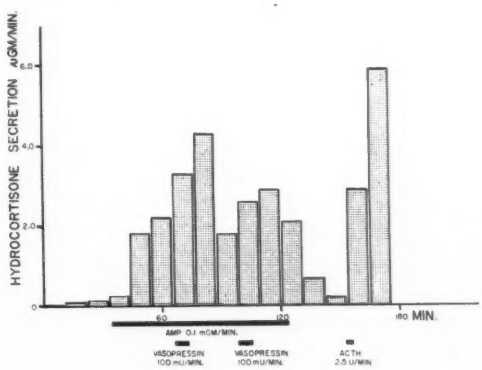

Figure 9

Comparison of the hydrocortisone-stimulatory activity of 3′,5′-adenosinemonophosphate with that of arginine vasopressin and ACTH in the hypophysectomized dog.

or greater than, that produced by ACTH. The stimulation of the secretion of cortisol after perfusion of the intact adrenal glands of our preparation with 3′,5′-AMP supports the

theory that corticosteroid synthesis is mediated by stimulation of the production of adrenal phosphorylase. Since the effect of vasopressin on the secretion of cortisol is relatively short-lived compared to that of 3′,5′-AMP and ACTH, it is conceivable that vasopressin may act, in some manner, to activate adrenal phosphorylase rather than actually to stimulate its production.

Discussion

In 1958 Royce and Sayers[6] demonstrated that vasopressin had an ACTH-like activity in the hypophysectomized rat. They suggested that vasopressin might directly stimulate the adrenal cortex but did not pursue this further. Instead, they proposed that it released ACTH from nonadenohypophyseal binding sites. It would appear that their first suggestion was correct. Our observations clearly demonstrate a direct cortisol-stimulating action of synthetic lysine, arginine and acetyl arginine vasopressins when they are perfused through the isolated adrenal glands. That this secretion is hydrocortisone was confirmed in 2 ways: (1) spectrophotometric analysis of the plasma Porter-Silber chromogens revealed maximal absorption at 410 mμ, similar to that of hydrocortisone alcohol, and (2) paper chromatography of methylene chloride extracts of plasma showed a motility identical to hydrocortisone alcohol (R.F. = 18).

Cortisol-stimulatory effects of arginine vasopressin, the naturally occurring form in dog and man, were noted with amounts as little as 7 mU. infused over a 7-minute period. Ginsburg and Brown[7] have demonstrated in the peripheral blood of rats amounts of ADH ranging from 1 to 26 mU./ml. under conditions of severe osmotic stimulation or hemorrhage. Such amounts are well within the range which, in our experiments, produces maximal secretion of cortisol.

Recently, it has been shown that as little as 2 mU. of arginine vasopressin will cause a release of ACTH when injected directly into the third ventricle of the dog.[10] Therefore, it is apparent that arginine vasopressin may have 3 major functions in salt and water homeostasis: (1) antidiuretic activity, (2) ACTH-releasing activity, and (3) direct stimulatory activity on the adrenal cortex.

We think it reasonable to ascribe an important role to arginine vasopressin in the "stress reaction." It is conceivable that the major role is played by the neurohypophysis, leaving to the adenohypophysis the regulation of nonstressful adrenal cortical homeostasis by means of ACTH.

Acknowledgment

Professor Vincent du Vigneaud's laboratory at Cornell University, School of Medicine, kindly supplied the synthetic lysine, arginine, and acetyl arginine vasopressins as well as the synthetic oxytocin used. Dr. Mary A. Root of the Lilly Research Laboratories generously donated crystalline glucagon.

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Discussion

Dr. Smith: I should like to ask Dr. Sawyer if he knows if, in the fishes generally, the hypophysis has migrated up to the floor of the diencephalon?

Dr. Sawyer: No, that is only true in the very primitive neurohypophysis of the lamprey. The lower diagram attempted to indicate that the neurohypophysis is completely surrounded by adenohypophysial tissue, and it comes down in most fishes—

Dr. Smith: No, that isn't my question. Do you know of any fishes in which the hypophysis as a whole remains on the roof of the mouth rather than migrating embryologically to the floor of the ventricle? This matter is of interest to me since Millot reports that in the coelacanth the hypophysis is located on the roof of the mouth, and has its primitive nerve supply and primitive vascular supply. For those interested in comparative anatomy and physiology—Dr. Sawyer did not remark on this, but perhaps he will—the absence of any known physiological action in extracts made from the so-called pituitary of the ascidians is somewhat against the thesis that a larval ascidian form was ancestral to the vertebrates.

Dr. Sawyer: It doesn't argue against it. It just doesn't support it.

Dr. Smith: I think it argues against it because if the gland were a pituitary, you ought to find something there.

Chairman Sayers: Dr. Hilton, when you set up experimental conditions in which you have a long-term infusion of the vasopressin, the cortisol secretion rises, and stays high?

Dr. Hilton: That's right.

Chairman Sayers: Do you have any comment on this point?

Dr. Hilton: Well, as far as ACTH is concerned, we can keep these glands secreting for 3 or 4 hours. We have never tried ACTH for any longer period. As to the transient effect of vasopressin, the only suggestion I have is that it is not stimulating a synthetic mechanism but it may be activating pre-existing renal phosphorylase or some similar compound.

Chairman Sayers: If there are no further questions, then I am going to call on Dr. Fishman to close the meeting. However, while he is coming up here to the podium, I think this is an appropriate time for the audience, the speakers, and the individual chairmen to express their appreciation to Dr. Fishman and to Dr. Pyles for the excellence with which they have planned and conducted this symposium. (Applause)

Dr. Fishman: There is nothing left to say except to thank the New York Heart Association for making such a meeting possible and to thank all of you for participating.

The Island of Knowledge

To the island of knowledge in the sea of ignorance, all that we can do is add a small bit to the periphery. But, when we do, we simply increase the boundary between the known and the unknown.—A. C. Zettlemoyer. *Molecular interactions with the surface of solids.* Am. Scient. **47**: 233, 1959.

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